JOURNAL OF AGRICULTURAL RESEARCH

VOL. 37 WASHINGTON, D. C., AUGUST 1, 1928 No. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

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INTRODUCTION

The continual increase in prevalence of tobacco ring spot in Virginia after it was first observed in 1917, together with the severe damage that it often caused to the tobacco crop, showed it to be a disease of great economic potentiality. These facts led to a study of the disease, the results of which were published in a preliminary report by Fromme, Wingard, and Priode[3] in 1927. In this report ring-spot was shown to be an infectious disease whose causative agent is apparently a virus.

The further study of the disease, reported in this paper, relates to its occurrence and symptoms on a variety of hosts other than tobacco, and on a number of species and varieties of Nicotiana.

REVIEW OF LITERATURE

In 1922 Fromme and Wingard,[4] and in 1924 Wingard and Godkin[5] reported ring-spot infection on some of the agronomic varieties of *Nicotiana tabacum* L. in Virginia. Such reports have also been made by the Plant Disease Survey from Kentucky and Ohio. In 1927, Fromme, Wingard, and Priode[6] reported four additional species of Nicotiana as being susceptible to infection, namely, *glutinosa*, *langsdorffii*, *paniculata*, and *sylvestris*; and the following varieties of *N. tabacum: Atropurpurea*, *auriculata*, *brasiliensis*, *calyciflora*, *colossea*, *gigantea*, *lacerata*, *latissima*, *macrophylla*, and *microphylla*. The agronomic varieties, Burley, Green's Wildfire Resistant, Little Orinoco, Macedonian, and Maryland, were also shown to be susceptible.

Priode,[7] in 1928, reported ring-spot infection on beet (*Beta vulgaris* L.), pokeweed (*Phytolacca decandra* L.), petunia (*Petunia hybrida* Vilm.), and New Zealand spinach (*Tetragonia expansa* Murr.).

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[1] Received for publication June 4, 1928; issued October, 1928. Paper No. 74 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

[2] The writer wishes to acknowledge his indebtedness to Dr. F. D. Fromme for many valuable suggestions in this study and in the preparation of the manuscript. For the seed of the different species and varieties of Nicotiana used in the experiments the writer is especially indebted to Dr. P. J. Anderson, of the Connecticut Agricultural Experiment Station, Dr. R. E. Clausen, of the University of California; Dr. James Johnson, of the University of Wisconsin; and Dr. W. D. Valleau, of the University of Kentucky.

[3] FROMME, F. D., WINGARD, S. A., and PRIODE, C. N. RING SPOT OF TOBACCO; AN INFECTIOUS DISEASE OF UNKNOWN CAUSE. Phytopathology 17: 321–328, illus. 1927.

[4] FROMME, F. D., and WINGARD, S. A. BLACKFIRE OR ANGULAR-LEAFSPOT OF TOBACCO. Va. Agr. Expt. Sta. Tech. Bul. 25: 16–18, illus. 1922.

[5] WINGARD, S. A., and GODKIN, J. TOBACCO DISEASES IN VIRGINIA AND THEIR CONTROL. Va. Agr. Col. Ext. Bul. 90: 25–27, illus. 1924.

[6] FROMME, F. D., WINGARD, S. A., and PRIODE, C. N. Op. cit.

[7] PRIODE, C. N. FURTHER STUDIES IN THE RING-SPOT DISEASE OF TOBACCO. Amer. Jour. Bot. 15: 88–93, illus. 1928.

Journal of Agricultural Research, Washington, D. C.

Vol. 37, No. 3
Aug. 1, 1928
Key No. Va. (Blacksburg)-5

14534—28——1

MATERIALS AND METHODS

The inoculum used throughout these studies was taken from a tobacco plant of the variety Kentucky Yellow obtained in Charlotte County, Va., August 20, 1926. The plant showed typical symptoms of ring spot on all of its leaves and as a result was considerably dwarfed. It was transplanted into the greenhouse bed at Blacksburg, where it continued to grow for over a year.

The ring-spot symptoms gradually failed to develop on the newly formed leaves, but the sap from the new growth continued to be infectious and would readily produce the disease on healthy plants. Infection was obtained on plants of *Nicotiana langsdorffii* Weinm. in December, 1926, and from then on transfers were made at regular intervals to healthy plants of *N. langsdorffii*, and *N. tabacum* in order to keep an abundant supply of disease on young vigorous plants.

Seed of many of the plants other than tobacco were obtained locally from growers and seedsmen, and many of the seeds were collected locally as seedlings and transplanted into the greenhouse beds. The plants in the majority of cases were grown in 4-inch pots which were sunk to about one-third of their depth in the soil of the greenhouse bed. In other cases, however, they were grown directly in the greenhouse bed soil. Plants, like tobacco, that make an erect growth were inoculated when they were 6 to 12 inches high.

The number of plants employed to represent the different hosts in the inoculation experiments varied from only 5 or 6 in a few cases to as many as 50 in others. In most cases the inoculations were repeated several times and at different stages in the development of the plant, new plants being used for each experiment, to make sure that the symptoms produced were typical. Material from 26 of the species of plants listed as hosts has been used as reinoculum on tobacco (*Nicotiana tabacum* L.), and typical ring-spot symptoms have developed in every instance. The species from which inoculum has been recovered and used to reinfect tobacco are indicated by an asterisk in the list of hosts given below. No attempt was made to obtain infection on tobacco from any of the other species, but there is no reason to believe that infection would not have been obtained on tobacco from these species had the inoculations been made.

The inoculum employed has consisted uniformly of the fresh sap and macerated tissues of heavily infected tobacco leaves, prepared by grinding the affected leaves in a mortar and adding enough tap or distilled water to dilute the sap to about 1 to 10. The inoculum was always applied immediately after being prepared in order to make sure of its virulency which disappears rapidly in expressed sap.

Infection has been transmitted readily by several methods of inoculation, but the method found to be most satisfactory and the one employed in this study is designated as the swabbing method. By this method the leaves of the trial plants are carefully swabbed with cotton plugs saturated with inoculum prepared as described above. Three or four leaves intermediate in size and age were inoculated on each plant. The inoculations were made late in the afternoon to avoid scorching of the leaves by the hot rays of the sun. No bell jars or covers of any kind were employed.

EXPERIMENTAL RESULTS

A wide selection of plants was inoculated in order to determine as far as possible the limits of the host range, and to study such variation in symptoms as might occur on different hosts. Especial attention was given to those plants which might be expected to carry the virus over winter in or near tobacco fields.

Infection was obtained on 38 genera of plants representing 17 families; namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae. The genera, species, and varieties of these families are listed below.

SOLANACEAE.—This family is well represented in the list of hosts, *Nicotiana acuminata* Grah., *N. clevelandii** [8] A. Gray, *N. glutinosa** L., *N. langsdorffii** Weinm., *N. longiflora* Cav., *N. paniculata* L., *N. plumbaginifolia** Viv., *N. quadrivalvis* Pursh, *N. quadrivalvis* var. *multivalvis* Gray, *N. repanda** W., *N. rustica* L. var. Iowa, var. English, and var. *jamaicensis*, *N. sanderae** (*Alta grandiflora* × *forgetiana*), *N. suaveolens* Lehm., *N. sylvestris** Speg., *N. tomentosa* Ruiz. and Pav., *N. trigonophylla* Dun., and *N. tabacum* L. developed infection. The following botanical varieties of *N. tabacum* also developed infection: *Atropurpurea, auriculata, brasiliensis, calycina, calyciflora, cavala, colossea, gigantea, lacerata, latissima, macrophylla, microphylla, purpurea, sanguinea*, and Turkish; and also the agronomic varieties, Adcock, Big Burley, Green's Wildfire Resistant, Kentucky Yellow, Little Orinoco, Lizard Tail, Macedonian, Maryland, Standup Burley, and Warne.

Other solanaceous plants found to be susceptible were *Datura stramonium** (Jimson weed), *Nicandra physalodes* (L.) Pers. (apple of Peru), *Petunia violacea** Lindl. (petunia), *Physalis angulata* L. (ground cherry), *Solanum carolinense** L. (horse nettle), *Solanum melongena* L. var. *esculentum* Nees (eggplant), *Solanum nigrum* L. (nightshade), and *Solanum pseudo-capsicum* L. (Jerusalem cherry).

CUCURBITACEAE.—Infection was readily obtained on the following representatives of this family: *Citrullus vulgaris* Schrad. (Monte Cristo watermelon), *Cucumis melo* L. var. *cantalupensis** Naud. (Honey Ball and Honey Dew cantaloupe), *Cucumis sativus** L. (Everbearing and Ideal White Spine cucumber), *Cucurbita pepo* L. var. *condensa** Bailey (Golden Summer Crookneck and Mammoth White Bush squash), *Cucurbita moschata** Duschene (Cushaw pumpkin), *Cucurbita pepo* L. (cornfield pumpkin), *Cucurbita pepo* L. var. *ovifera** Bailey (Nest Egg gourd), *Lagenaria leucantha* Rusby (Dipper gourd), and *Luffa cylindrica** Roem. (Dish Cloth gourd).

COMPOSITAE.—The list of ring-spot hosts in this family includes both weeds and cultivated plants, namely, *Ambrosia artemisiifolia* L. (small ragweed), *Ambrosia trifida* L. (giant ragweed), *Aster laevis* L. (Ostrich Plume aster), *Bidens discoidea* (T. and G.) Britton (Spanish needle), *Calendula officinalis** L. (pot marigold), *Callistephus chinensis* Nees (China aster), *Erigeron canadensis* L. (field erigeron), *Helianthus annuus* L. (Mammoth Russian sunflower), *Lactuca sativa* L. var. *capitata** L. (head lettuce), *Lactuca scariola* L. (prickly lettuce),

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[8] Infection has been obtained on tobacco with an inoculum taken from the species that are marked with an asterisk.

Tagetes erecta L. (African marigold), and *Zinnia elegans** Jacq. (garden zinnia).

LEGUMINOSAE.—A number of species in this family have been shown to be susceptible to ring-spot infection, namely, *Dolichos lablab* L. (Broad Windsor bean), *Melilotus officinalis** Lam. (sweet clover), *Phaseolus lunatus* L. (small Lima or Sieva bean), *Phaseolus vulgaris** L. (kidney bean), and *Vigna sinensis* Endl. (black-eye cowpea).

The families listed below are represented by one or more host species as indicated.

CHENOPODIACEAE.—*Beta vulgaris** L. (garden beet and Silesian sugar beet), *Beta vulgaris* L. var. *cicla* L. (Swiss chard), *Chenopodium album* L. (lamb's-quarters).

VIOLACEAE.—*Viola papilionacea* Pursh (common violet), and *Viola tricolor* L. (giant pansy).

AIZOACEAE.—*Tetragonia expansa* Murr. (New Zealand spinach).

AMARANTHACEAE.—*Amaranthus paniculata* L. (pigweed).

CONVOLVULACEAE.—*Ipomoea purpurea* (L.) Roth. (morning-glory).

CRUCIFERAE.—*Barbarea Barbarea** (L.) MacM. (winter cress).

DIPSACEAE.—*Scabiosa atropurpurea* L. (sweet scabious).

EUPHORBIACEAE.—*Ricinus communis* L. (castor-oil plant).

LABIATAE.—*Salvia splendens* Ker. (scarlet sage).

MALVACEAE.—*Hibiscus esculentus* L. (okra).

PHYTOLACCACEAE.—*Phytolacca decandra** L. (pokeweed).

POLYGONACEAE.—*Polygonum hydropiper* L. (smartweed).

SCROPHULARIACEAE.—*Antirrhinum majus** L. (snapdragon).

In reading the list of host plants given above one is likely to conclude that the ring-spot virus is not very specific in its ability to infect. Such, however, does not seem to be the case, since many plants that failed to develop symptoms of any kind have been inoculated in these experiments. Of course, some of these may prove to be susceptible to infection when subjected to further experimentation, but in that event the number should be small.

No infection was obtained on the following plants: *Althaea rosea* Cav. (hollyhock), *Berberis vulgaris* L. (barberry), *Brassica alba* Rabenh. (mustard), *Brassica napobrassica* Mill. (rutabaga), *Brassica rapa* L. (turnip), *Brassica oleracea* L. var. *capitata* L. (cabbage), *Brassica oleracea* L. var. *acephala* DC. (collards), *Bryophyllum pinnatum* Kurz. (bryophyllum), *Capsicum frutescens* L. var. *grossum* Bailey (bell pepper), *Centranthus ruber* DC. (Jupiter's beard), *Chrysanthemum carinatum* L. (chrysanthemum), *Cirsium arvense* (L.) Scop. (Canada thistle), *Coleus blumei* Benth. var. *verschaffeltii* Lem. (coleus), *Cosmos diversifolius* Otto Ortg. (cosmos), *Dahlia merckii* Lem. (dahlia), *Dactylis glomerata* L. (orchard grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Dianthus barbatus* L. (sweet William), *Glycine hispida* Max. (soy bean), *Lycopersicon esculentum* Mill. (tomato), *Malconia maritima* R. Br. (stock), *Mentha piperita* L. (mint), *Oxalis repens* Thunb. (wood sorrel), *Pastinaca sativa* L. (parsnip), *Pisum sativum* L. (peas), *Phleum pratense* L. (timothy), *Pelargonium domesticum* Bailey (geranium), *Plantago major* L. (plantain), *Pyrus malus* L. (apple), *Rosa* spp. (rose), *Rumex patientia* L. (dock), *Rumex abyssinicus* Jacq. (rhubarb), *Raphanus sativus* L. (radish), *Solanum dulcamara* L. (bittersweet), *Solanum tuberosum* (potato), *Stellaria media* (L.) Cyrill. (chickweed), *Spinacea oleracea* L. (spinach), *Tragopogon porrifolius* L. (salsify), *Tradescantia flu-*

minensis Vill. (tradescantia), *Triticum vulgare* Vill. (wheat), *Tropaeolum majus* L. (nasturtium), and *Zebrina pendula* Schnizl. (Wandering Jew).

LEAF SYMPTOMS OF THE DISEASE

The symptoms of ring spot are restricted to the leaves of most of the host plants, but they may also appear on the stems and fruits in some instances, as will be described later. The symptoms vary considerably on different hosts, but still show a remarkable similarity in

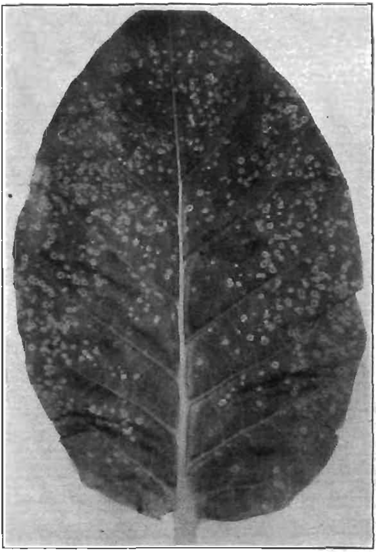


FIG. 1.—Ring-spot infection on leaf of Turkish tobacco four days after inoculation. Natural size

practically all cases. Infection on the leaf usually results in the development of rings and lines that spread out in a zigzag manner, and these are the most characteristic symptoms of the disease.

Since the symptoms vary somewhat in even the most closely related plants, it might appear advisable to describe them in detail for each host. This procedure, however, is readily seen to be impractical when the large number of susceptible plants is considered. Therefore, instead of treating each host separately, those which show strikingly similar symptoms will be grouped together with a description of the symptoms that are characteristic of the group.

GROUP 1

The symptoms of the ring-spot disease are quite similar for the following varieties of *Nicotiana tabacum: Atropurpurea, auriculata, brasiliensis, calycina, calyciflora, cavala, colossea, lacerata, latissima, macrophylla, microphylla, purpurea, sanguinea,* and Turkish; and also for the agronomic varieties, Adcock, Little Orinoco, Lizard Tail, Green's Wildfire Resistant, Kentucky Yellow, Macedonian, Maryland, and Warne. The symptoms occur only on the leaves of this group of plants, and are first evident within 48 to 60 hours after inoculation.

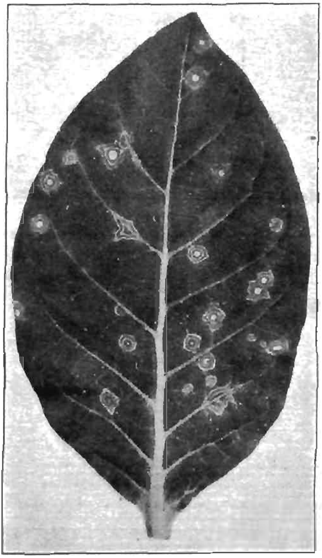


FIG. 2.—Ring-spot infection on Turkish tobacco leaf eight days after inoculation. Note the difference in type of spot produced on the intervein tissue and that along the veins. Natural size

The initial spot consists of a small ring of necrotic tissue surrounding an island of apparently normal tissue. (Fig. 1.) The primary rings are usually not more than 1 mm. in diameter, and in some cases they are so small that they appear as mere dots. The necrotic rings or margins of the young spots appear as translucent lines in transmitted light. They become blanched or brown in color in a few days after they appear. A secondary ring or margin, 3 to 4 mm. in diameter, appears in four days (fig. 2); and a third one, 6 to 7 mm. in diameter, in about five days. This process continues when the spots are thinly distributed over the leaf until finally there are several of these necrotic rings with alternating zones of normal-appearing tissue, as in Figure 3. The center of the spot consists of the primary ring and its island of encircled tissue. The tissue in the center of the spot often dies in five or six days after the spot appears, especially if the primary ring is very small in diameter. In such cases the center of the spot has the appearance of a small dot.

The outlines of the spot vary according to the location. They are circular, as described above, when centered on intervein tissue, but when centered on the larger veins and midrib they are very irregular in outline. (Figs. 2, 4, and 5.) The infection follows the vein and

its branches, and the outline of the spot often suggests that of a deeply lobed leaf. (Figs. 4 and 5.)

Infection becomes systemic in 8 to 14 days after inoculation. If some of the leaves of a medium-sized plant are inoculated, local symptoms will appear in 48 to 60 hours, and in about 6 days symptoms will begin to appear on the uninoculated leaves, especially on the upper ones. The infection will continue up the plant, and in 10 to 14 days all the tiny bud leaves will show typical ring-spot symptoms. The symptoms of systemic infection show a marked tendency to follow the midrib and larger veins of the leaves, yet the characteristic circular spots appear on the intervein tissue. (Fig. 5.) As soon as the infection becomes systemic and symptoms appear on all the leaves of the plant—in about 10 to 14 days after inoculation—new leaves will appear on which symptoms may either fail to develop altogether or will develop only slightly, appearing only on the tip half of the leaf. The symptoms gradually fail to develop on the new growth, and those that do develop are located nearer and nearer the tips of the leaves until finally only faint symptoms can be detected at the very tip of the leaf. (Fig. 6.) New leaves produced after this stage

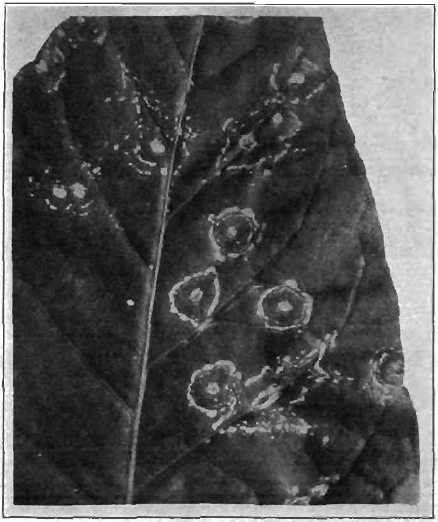


FIG. 3.—Leaf of Green's Wildfire Resistant tobacco showing ring-spot infection three weeks after inoculation. Natural size

has been reached may appear normal, or they may show a faint grayish mottling. In some cases these apparently normal leaves on affected plants develop numerous brown necrotic specks. The margins are necrotic in the first spots that appear on these plants, but as new leaves appear and as the symptoms begin to become less conspicuous the margins of the spots are no longer necrotic but are chlorotic or only of a lighter green color than the normal tissue.

The leaves of plants on which the ring-spot symptoms have become masked appear to be a little thicker and to have more of a leathery

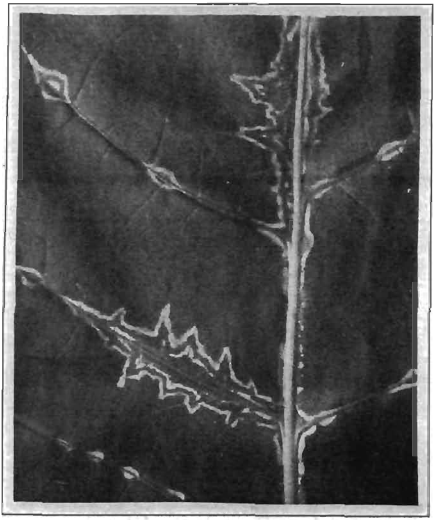


FIG. 4.—Symptoms of ring spot on a leaf of *Nicotiana tabacum* var. *purpurea* as a result of systemic infection. Natural size

texture than those of normal plants. (Fig. 7.) The leaves of suckers that develop from these old plants usually appear normal or show only a grayish mottling. However, typical ring-spot symptoms have appeared on the leaves of such suckers in a few instances. Several attempts have been made to produce ring-spot symptoms on the young leaves of both suckers and plants that have reached the stage of masked symptoms by reinoculating them with virus from severely affected leaves, but the results have been negative in every instance. On the other hand, the juice from these apparently healthy leaves readily produces typical symptoms when used to inoculate healthy plants. This masking of symptoms, or development of immunity, or

whatever it is, seems to hold under greenhouse conditions for practically all the plants tested.

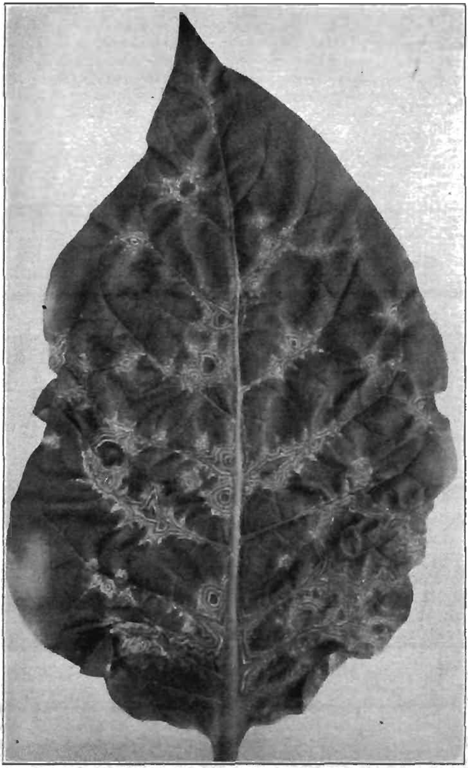


FIG. 5.—Systemic ring-spot infection on a leaf of Turkish tobacco. The lower leaves on the plant from which this leaf came were inoculated five weeks before this leaf was photographed. Natural size

GROUP 2

This group is represented by *Nicotiana rustica* var. English, *N. tabacum* var. *gigantea*, and the Burley strains of *N. tabacum*, namely,

Big Burley and Stand-up Burley. The ring-spot symptoms begin to appear on the leaves of these plants in about three days after inoculation. The first sign of infection is the development of a small necrotic spot which is surrounded by a definite light yellow halo. (Fig. 8.) The spots at this stage look almost exactly like wildfire spots, caused by *Bacterium tabacum* Wolf and Foster. The center of the spot may begin as a small brown speck or it may at first be composed of a small ring of necrotic tissue surrounding an island of apparently normal tissue. The tissue within the ring becomes chlorotic and then necrotic, giving the spot its characteristic center. The necrotic centers, 1 to 5 mm. in diameter, are surrounded by a definite light

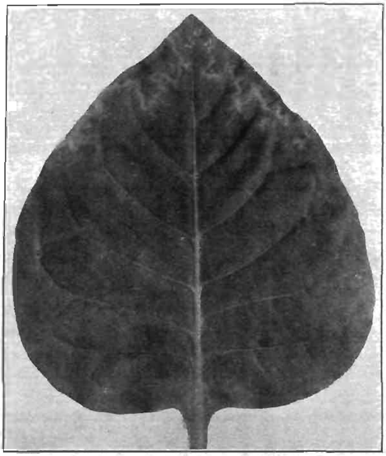


FIG. 6.—One of the top leaves of a Turkish tobacco plant showing the last trace of ring-spot symptoms on its tip. Lower leaves from the same plant were severely affected. Slightly reduced

green halo, at the margin of which a broken or a continuous line of necrotic tissue develops. This ring soon becomes surrounded by a light green halo, which in turn develops a necrotic line at its margin. This process continues until several such rings are developed and the spot measures 10 to 20 mm. in diameter. Numerous fine specks often develop in the alternating zones of living tissue.

Infection becomes systemic in 10 to 14 days and the symptoms begin to appear on the bud leaves. The leaves of intermediate size develop circular necrotic spots, or lines of necrotic tissue surrounding circular islands of living tissue, and zigzag lines of necrotic tissue running at random in the intervein tissues. (Fig. 9.) The young bud leaves are often almost completely destroyed. Big necrotic spots

appear and the leaf becomes twisted and distorted and never makes much growth. Another characteristic symptom on the leaves of Burley tobacco is the development of irregular lines of necrotic tissue

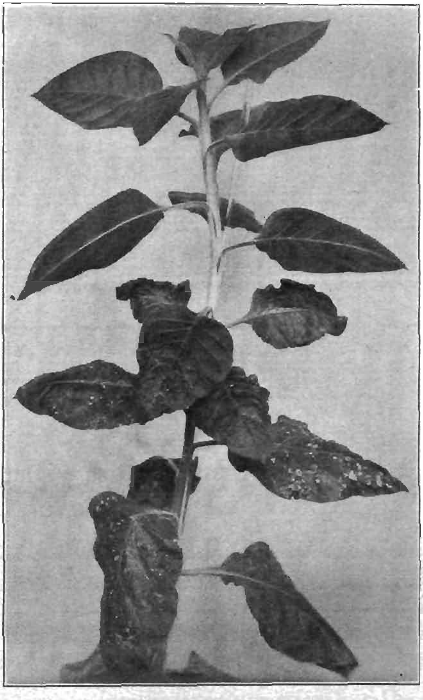


FIG. 7.—Turkish tobacco plant 23 days after inoculation with ring-spot. Note the gradual decline in the development of ring-spot symptoms on the upper leaves until finally the top leaves appear perfectly normal. Much reduced

along the midrib and larger veins as shown in Figure 10. There is a marked dwarfing of the entire plant as a result of infection.

The plants in this group also, sooner or later, develop an apparent immunity or tolerance to the disease. New leaves develop on which no symptoms appear, and yet the sap from such leaves is quite virulent when used as inoculum on healthy plants.

GROUP 3

This group includes pokeweed, petunia, *Nicotiana langsdorffii*, and *N. rustica* var. Iowa and var. *jamaicensis*. The symptoms of infection on these plants are similar in many respects to those of the plants in Group 1. The spots appear in about three days after inoculation

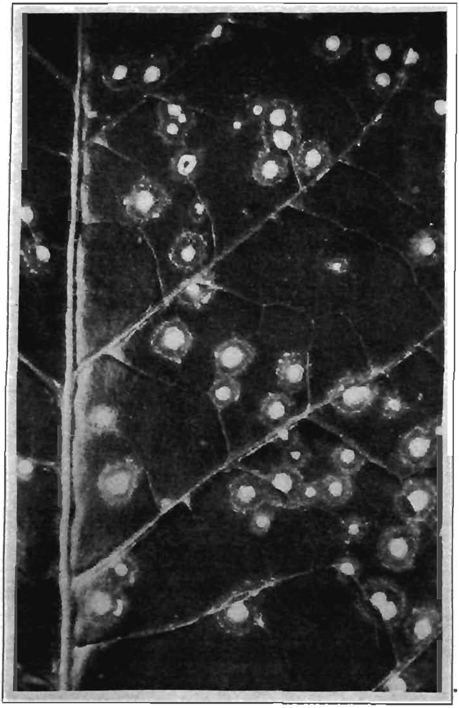


FIG. 8.—Ring-spot infection on leaf of Big Burley tobacco 10 days after inoculation. About natural size

,nd are composed of an island of normal green tissue surrounded by . continuous line of necrotic tissue which at first has a water-soaked ppearance, but after a few days dries out and becomes perfectly hite, as shown in Figure 11. The spots on pokeweed usually agree

with the above description, but in some cases they are more compact and the marginal lines instead of being composed of continuous lines of necrotic tissue are made up of a series of necrotic specks. (Fig. 12.)

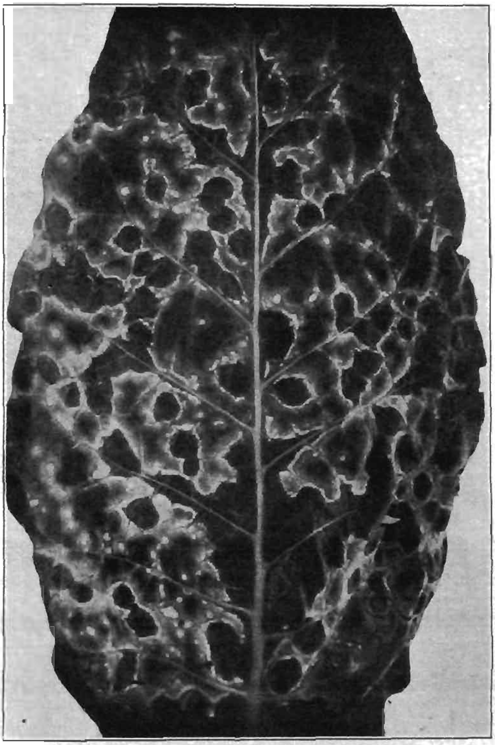
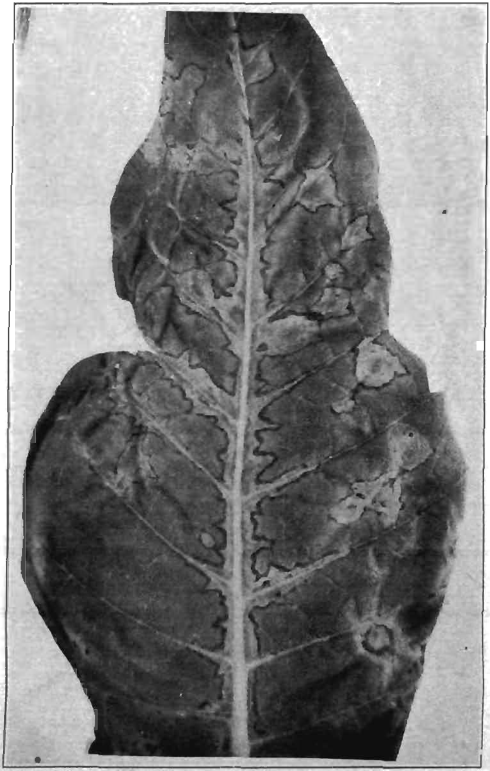


FIG. 9.—Systemic ring-spot infection on one of the top leaves of a Big Burley tobacco plant six weeks after some of the lower leaves had been inoculated. Slightly reduced

The primary rings are followed in two or three days by secondary rings of the same type and the secondary rings are in turn followed by tertiary ones. The tissue intervening between the necrotic mar-

gins retains its normal green color. Infection becomes systemic and for a time the new leaves will develop the typical spots, but after two to four weeks new leaves form on which the symptoms develop only faintly with the marginal lines no longer becoming necrotic. This continues until finally no symptoms appear on the new growth. The old spots on pokeweed leaves become masked by a thin velvety growth which is of a Tyrian rose color.



GROUP 4

This group is represented by a wide variety of plants, all of which seem to be hypersensitive to the ring-spot virus. It includes the following species of Nicotiana: *Clevelandii, glutinosa, longiflora, multivalis, plumbaginifolia, quadrivalis, repanda, sanderae, suaveolens, sylvestris*, and *trigonophylla;* also garden aster, China aster, kidney bean, Lima bean, Broad Windsor bean, castor-oil bean, eggplant, water-

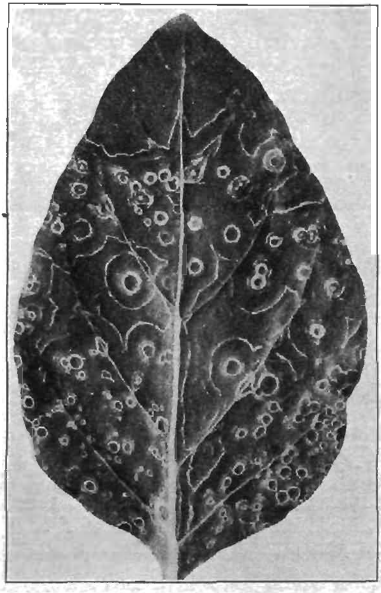


FIG. 11.—Ring-spot infection on leaf of *Nicotiana langsdorffii* three weeks after inoculation. About two-thirds natural size

melon, Jimson weed, prickly lettuce, horse nettle, New Zealand spinach, ground cherry, and snapdragon.

The most characteristic symptom of infection for this group is the development of spots that are necrotic throughout. The primary infection on certain of these plants, namely, *Nicotiana sylvestris, N. repanda, N. glutinosa*, Lima bean, garden bean, castor-oil bean, cowpea, watermelon, and horse nettle, appears as definite necrotic spots in three days after inoculation. The spot has a light center about 1 mm. in diameter (figs. 13 and 14) which is surrounded by a band

of dark brown tissue. The margin of the spot consists of newly killed tissue which has a black, water-soaked appearance. This marginal tissue turns brown on drying and a new necrotic band is formed. Thus the spots enlarge until they finally coalesce and either kill the entire leaf or large sections of it. (Fig. 15.) The initial symptoms on certain other plants of this group, of which *N. sanderae*, *N. multivalvis*, snapdragon, and New Zealand spinach are typical examples, are typical rings as described for plants in Group 1. (Fig. 16.) The spots remain as alternating zones of living and necrotic tissue for about a week and then the entire spot becomes necrotic and the necrosis spreads until the spots coalesce and kill the entire leaf or large areas thereof. (Fig. 15.) The original necrotic lines can still be seen in the necrotic spots on this leaf.

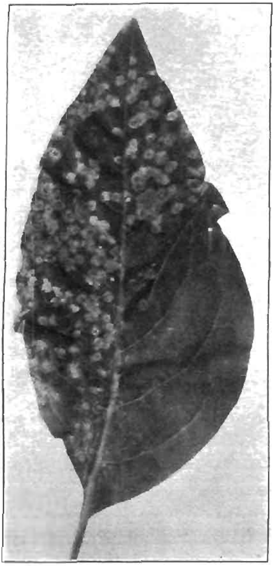


FIG. 12.—Systemic ring-spot infection on pokeweed leaf. Natural size

The foregoing relates to symptoms that develop on the parts of the leaf to which the inoculum was applied. After infection has become systemic in these plants, spots develop on the new leaves that are composed of rings of necrotic tissue intervening between zones of apparently normal tissue. (Fig. 17.) Finally, the symptoms fail to appear on the new growth.

GROUP 5

This group is represented by cantaloupe (Honey Ball and Honey Dew), cucumber (Everbearing and Ideal White Spine), gourd (Nest Egg, Dipper, and Dish Cloth), pumpkin (Cornfield and Cutshaw), and squash (Golden Summer Crookneck and Mammoth White Bush). These plants are very susceptible to ring-spot infection, as shown by the abundant development of symptoms within three days after inoculation. The young spots on the leaves consist of a small yellow to brown pin-pointlike center surrounded by a light-yellow margin or halo. (Fig. 18.) The pin-point center in some cases becomes necrotic, but as a rule the necrosis does not spread throughout the spot. Although the type of spot described above is the most typical for this group of plants, there is still a tendency for definite rings to appear, especially on the leaves to which the inoculum is

applied. In some cases the tiny brown centers are surrounded by two or three necrotic rings.

Infection becomes systemic in about 10 days and the halo type of spot appears in great numbers on all the new leaves. (Fig. 18.) In the case of the dipper gourd the leaf is literally covered with light-yellow spots no more than 1 mm. in diameter. These tiny spots have definite pin-pointlike centers that are water soaked in appearance. On squash and Nest Egg gourd the spots are much larger (figs. 18 and 19) but are of the same type. On pumpkin the spots are

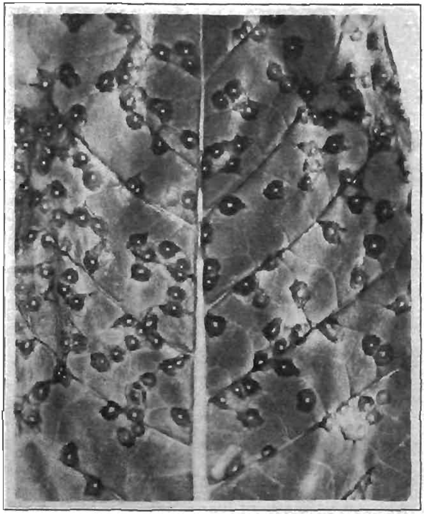


FIG. 13.—Ring-spot infection on leaf of *Nicotiana sylvestris* 10 days after inoculation. Note the light centers and brown margins of the spots. Natural size

restricted primarily to the leaf veins. (Fig. 20.) The halo type of spot also appears on cantaloupe, cucumber, and Dish Cloth gourd; but in addition to this definite rings and zigzags often develop. The lines and zigzags are much lighter in color than the normal green leaf tissue but as a rule there is no necrosis.

GROUP 6

This is a miscellaneous group in which all the remaining host plants have been placed, even though their ring-spot symptoms may differ slightly from each other. In general, infection on these hosts

produces either the ringlike spot or the fine zigzag lines that run at random across the leaf. There is not sufficient difference in the symptoms on these plants to warrant separating them into main groups, but they will be placed in subgroups according to their ring-spot symptoms.

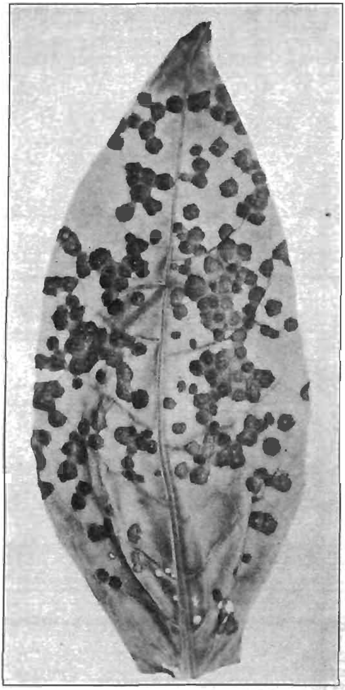


FIG. 14.—Ring-spot infection on leaf of *Nicotiana repanda* two weeks after inoculation. Note the brown necrotic spots. Natural size

Garden beet, sugar beet, Swiss chard, pot marigold, and *Nicotiana tomentosa* develop very faint zigzag lines on their leaves as a result of ring-spot infection. These lines are of a lighter color than the normal tissue but are never necrotic.

Infection on *Nicotiana acuminata*, pigweed, lamb's-quarters, field erigeron, morning-glory, winter cress, small ragweed, giant ragweed, violet, giant pansy, sweet scabious, night shade, apple of Peru, and sweet clover produces definite necrotic rings with the tissue within the ring usually remaining green.

On Spanish needle, African marigold, and smartweed the symptoms develop as very fine zigzag lines which may or may not form inclosures with irregular borders. This type of infection is shown in Figure 21. The tissue in the marginal lines becomes necrotic and turns white.

Infection on Jerusalem cherry results in the formation of both rings and zigzags on the leaves. (Fig. 22, A.) The rings and zigzags are made up of chlorotic tissue which never becomes necrotic. On scarlet sage definite light-green lines develop along the midrib and larger veins of the leaf. This is especially true of the symptoms that develop on this plant as a result of systemic infection. Infection on sunflower leaves produces a large circular spot, composed of a

center of normal green tissue surrounded by a broad band of light green. This light green border becomes necrotic with age and takes

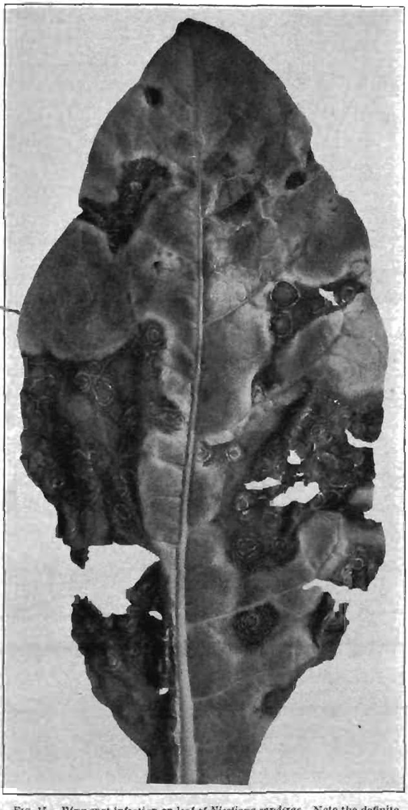


FIG. 15.—Ring-spot infection on leaf of *Nicotiana sanderae*. Note the definite concentric rings which are later followed by a complete necrosis of all the surrounding tissues. Four-fifths natural size

on a brown color. Some of the spots are a centimeter or more in diameter. Infection becomes systemic and produces brown necrotic

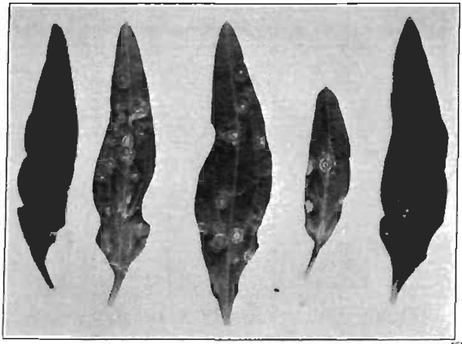


FIG. 16.—Ring-spot infection on snapdragon leaves a week after inoculation. Natural size

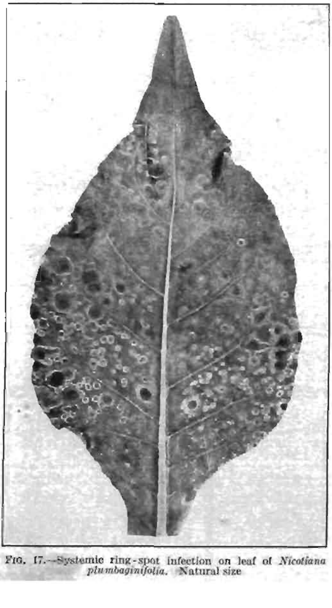


FIG. 17.—Systemic ring-spot infection on leaf of *Nicotiana plumbaginifolia*. Natural size

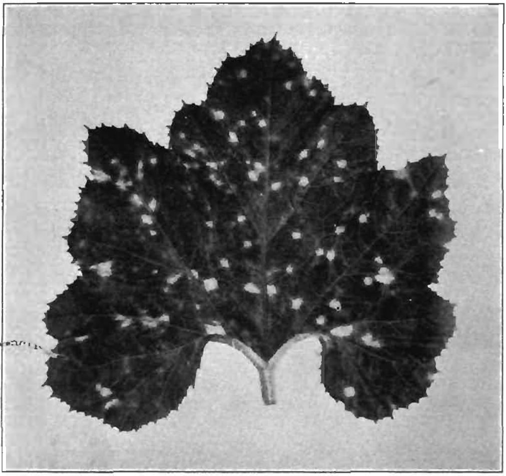


FIG. 18.—Systemic ring-spot infection on leaf of Golden Summer Crookneck squash. Natural size

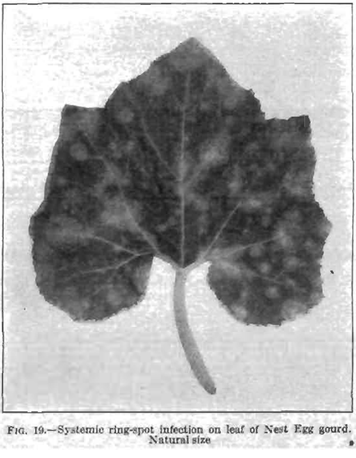


FIG. 19.—Systemic ring-spot infection on leaf of Nest Egg gourd. Natural size

rings on the involucral bracts of the flower. The symptoms on okra leaves are similar to those on sunflower except that the margins of the spots never become necrotic. No definite ring symptoms develop on the leaves of zinnia and head lettuce, and yet infection becomes systemic in these plants. The new leaves that develop after these plants have been inoculated show a marked stunting and in some cases are slightly crinkled but no other symptoms have been observed. The juice from such leaves, however, produces typical ring-spot symptoms when used as inoculum on Turkish tobacco.

STEM SYMPTOMS OF THE DISEASE

As a rule, symptoms do not occur on the stem of the host plant as a result of ring-spot infection; but in exceptional cases the stem

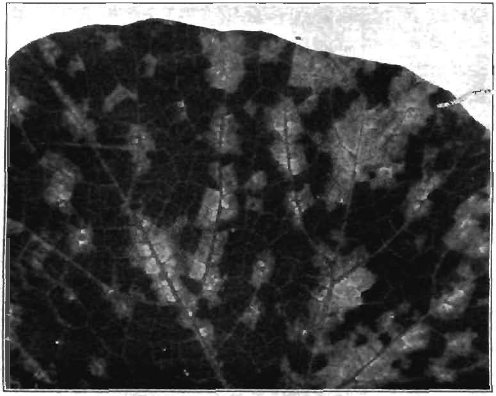


FIG. 20.—Systemic ring-spot infection on leaf of cornfield pumpkin. Natural size

shows marked symptoms and the entire plant may be killed. Stem lesions are most likely to occur on plants that are hypersensitive to the ring-spot virus, such as those listed in Group 4.

Representatives of the following plants have been killed outright in one or more of the inoculation experiments: *Nicotiana clevelandii*, *N. glutinosa*, *N. longiflora*, *N. multivalvis*, *N. plumbaginifolia*, *N. quadrivalvis*, *N. sanderae*, and *N. sylvestris*. The symptoms first appear on the leaves as dark circular necrotic spots. Then dark sunken lesions develop on the leaf petioles, and in about 10 days similar lesions appear on the main stem of the plant, in some cases extending its full length. The plant becomes twisted and dies in two or three weeks. The vascular tissues of the stem and roots become black before the plant dies. One of the *N. glutinosa* plants was

killed only to the ground line and later sent up suckers. This is very unusual, however, because the roots are usually destroyed in cases of such severe infection.

As noted, the above-named plants may or may not be killed outright by the ring-spot infection; but the following species are apparently always killed outright: Snapdragon, kidney bean, Lima bean,

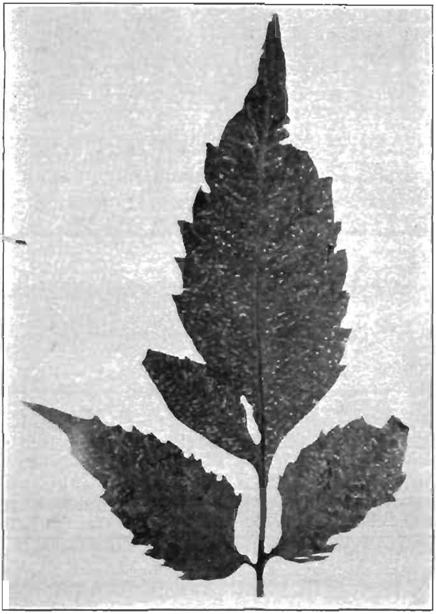


FIG. 21.—Ring-spot infection on leaf of Spanish needle. Note the very fine lines on this leaf. About natural size

Broad Windsor bean, cowpea, and New Zealand spinach. The necrotic spots that appear on the leaves of these plants are followed in two or three days by an infection of the leaf veins and petioles. The veins turn black and shrivel, and the lesions on the petioles become dark and sunken; and in the case of kidney bean, the infection looks very much like that of anthracnose. Dark sunken lesions also appear on the main stem, and the plants die in 10 to 14 days.

FRUIT SYMPTOMS OF THE DISEASE

Symptoms of ring-spot infection have recently been obtained on the fruits of some of the hosts; and, so far as the writer knows, this

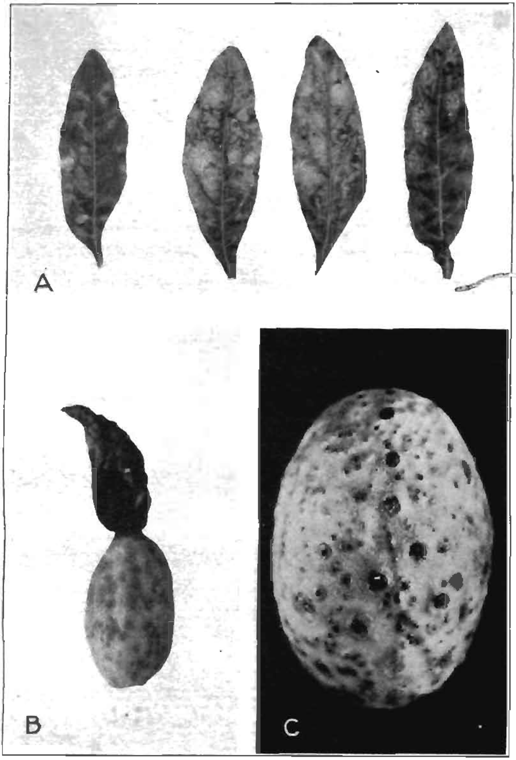


FIG. 22.—A, systemic ring-spot infection on the leaves of Jerusalem cherry. B, ring-spot infection on a young Nest Egg gourd; infection came from the vine. C, mature fruit of Nest Egg gourd showing ring-spot symptoms as a result of systemic infection in the plant on which it was produced; note the concentric rings of the spots. All natural size

s the first time the symptoms have ever been observed on fruits. These observations were made on the fruits of Nest Egg gourd and Golden Summer Crookneck squash, which were grown in the green-

house. The leaves of these plants were inoculated with the ring-spot virus when the plants were about 2 weeks old. Infection became systemic, and all the new leaves developed typical symptoms; and when the young fruits appeared they also showed the characteristic ring-spot symptoms.

The fruits show the ring-spot symptoms very early and often drop when they are only 1 or 2 inches in length. The symptoms first appear as small, circular, water-soaked spots not more than 1 mm. in diameter. (Fig. 22, B.) They become depressed in a few days and give the surface of the fruit a pitted appearance. (Fig. 23.) These pits become encircled by a very narrow line of water-soaked tissue in four or five days. These circular margins or rings are more easily seen when thin horizontal sections are cut from the surface of the fruit. The spots penetrate to a depth of 2 to 3 mm. as a rule, and in some cases can be traced all the way to the seed cavity. The spots develop a deep green pigment in contrast to the white or yellow pigment of the normal tissue. (Fig. 22, C.) Three of the infected Nest Egg gourd fruits grew to maturity, and the spots which had at first been depressed gradually became elevated and finally appeared as pimples on the surface of the mature fruit. (Fig. 22, C.) The spot in its final stage is composed of a small elevated center surrounded by one or more definite rings.

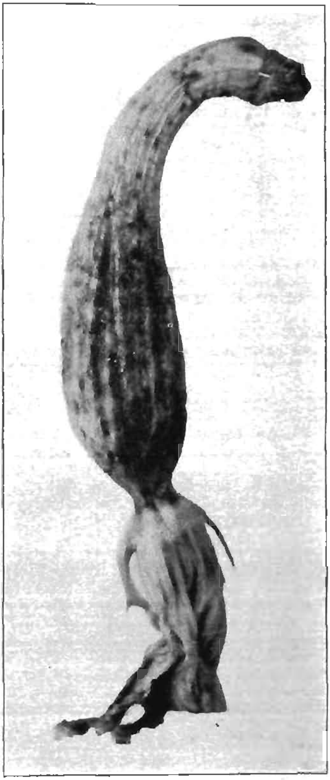


FIG. 23.—Ring-spot infection on a young Golden Summer Crookneck squash. Infection was systemic in the plant on which this fruit was produced. Natural size

DISCUSSION OF RESULTS

The results of the inoculation experiments show that the ring-spot virus has a very extensive host range. They also show that the virus is very specific in its infective properties, since so many of the plants that were tested failed to develop infection, and especially the solanaceous plants, pepper, potato, and tomato. One should naturally expect plants that are so closely related to tobacco to be susceptible. Repeated attempts were made to infect these plants but the results were always negative.

The list of susceptible plants includes horse nettle, pokeweed, and sweet clover, all of which may play an important part in the overwintering of the ring-spot virus. Horse nettle is a common weed in tobacco fields throughout Virginia, and it may serve as a constant source of infection.

Pokeweed is common in old plant-bed sites and around the borders of tobacco fields. This plant is very susceptible to ring-spot infection, and the sap from its infected leaves is very infectious on tobacco. Infection becomes systemic in this plant, but the symptoms fail to appear on the new growth a short time after the plant becomes infected. It appears possible, therefore, that apparently normal pokeweed plants may carry the virus and act as a reservoir of inoculum for tobacco.

These results also help to explain why ring-spot is usually present on tobacco that is grown in old garden and building sites. Pokeweed, Jimson weed, and horse nettle are always in evidence around such places and, if infected, may serve as a source of infection for tobacco.

Ring-spot infection is often very severe on tobacco crops that follow clovers and alfalfa in rotation. Such fields have been observed in which 50 per cent of the plants were heavily affected with ring-spot. These observations suggested that the leguminous crops were in some way responsible for the infection on tobacco, especially when sweet clover was found to be very susceptible to infection by the ring-spot virus. Natural infection has recently been found rather commonly on sweet clover that is growing on the experiment station plots, and in rotation on the college farm. Inoculum from both the artificially inoculated and the naturally infected sweet-clover plants readily produced the typical ring-spot symptoms on tobacco. Other clovers are no doubt susceptible to infection. These results help to account for infection on tobacco following clover in rotation.

The virus loses its potency at ordinary temperatures as soon as the infected plant tissue decays or becomes thoroughly dry, and in the expressed sap in about 24 hours. It seems, therefore, that the virus must overwinter in biennial or perennial plants, or in the embryo of infected seed. Since the virus appears to retain its potency indefinitely regardless of temperature as long as it is contained in living tissue, it seems that the embryo of the seed should carry it through the winter months. The systemic nature of infection should provide ample opportunity for the seed to become infected. The lesions on the young squash and gourd fruits penetrate the wall of the ovary and may extend to the placental tissue. Embryonic seeds might possibly become infected by their proximity to infected tissue.

Plants seem to develop an immunity to the ring-spot disease. Symptoms appear in abundance when the plants are inoculated and infection soon becomes systemic, as shown by the development of symptoms on the leaves that were uninoculated. New growth takes place and the ring-spot symptoms will appear on it. This process continues for 10 days to 2 weeks and then symptoms appear only faintly on the new leaves and suckers. Finally, the symptoms fail to appear on the new growth and the leaves appear normal. Attempts have been made to produce symptoms on these leaves by reinoculation with virus from heavily infected leaves from other plants, but without success. The sap from these apparently normal leaves is just as virulent as that from leaves that show the disease symptoms, indicating that the virus is present in such leaves even though the disease symptoms fail to appear.

SUMMARY

The ring-spot virus is capable of producing disease symptoms, more or less typical, on a wide variety of plants.

Plants of 72 genera have been inoculated, 38 of which developed infection, representing 17 families, namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae.

The first symptoms appear in about 3 days after inoculation and infection becomes systemic about 10 days later.

The symptoms of the disease are restricted to the leaves of the majority of the plants, but they may also appear on the stems and fruits of some of the hosts.

Symptoms appear on the fruits of Nest Egg gourd and Golden Summer Crookneck squash as a result of systemic infection of the plant.

Certain plants are often killed outright as a result of ring-spot infection, namely, *Nicotiana clevelandii*, *N. glutinosa*, *N. longiflora*, *N. multivalvis*, *N. plumbaginifolia*, *N. quadrivalvis*, *N. sanderae*, *N. sylvestris*, Broad Windsor bean, Black-eye cowpea, kidney bean, Lima bean, snapdragon, and New Zealand spinach.

Infection has been found occurring naturally on sweet clover and the commercial varieties of tobacco.

Tobacco (*Nicotiana tabacum*) has been infected with virus recovered from the following plants: *N. clevelandii*, *N. glutinosa*, *N. langsdorffii*, *N. plumbaginifolia*, *N. repanda*, *N. sanderae*, *N. sylvestris*, Jimson weed, garden petunia, horse nettle, cantaloupe, cucumber, squash, pumpkin, gourd, calendula, head lettuce, garden zinnia, kidney bean, garden beet, winter cress, pokeweed, snapdragon, and sweet clover.

BACTERIAL POCKET DISEASE OF THE SUGAR BEET[1]

By Nellie A. Brown

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INTRODUCTION

While the intensive work with crown gall on various hosts was in progress some years ago, galls on sugar beets received attention and requests were sent to beet growers for sugar beets showing any sort of excrescence. In 1910 sugar beets were received from Holly, Colo., having at the crown definite galls, which were thought to be crown galls. Many of these did not show the typical features of crown gall, most of the outgrowths being deeply indented nodules, but because they were definite tumors at the crown no question as to their nature was raised at the time. Some of the galls were more or less globose and looked much like crown gall, but many were made up of numbers of small nodules, which grew around the crown and looked like cultivation wounds.

When the galls were cut across, a condition unlike crown gall was noted. There were brown areas inside instead of sound white tissue, and surface lesions from which the discoloration could be traced to the interior. Some of the tumors were fairly smooth on the outside and had no visible breaks in the surface, but when they were cut across, large brown areas, which could be traced for some distance in the interior, were seen. In every cut gall the discoloration could be traced from the outer part to the inner, where it became lost in sound white tissue.

Frequently the edges of the white tissue adjacent to the brown watery places were discolored red or purple. When a cut was made through the brown tissue a mucilaginous substance often oozed out. All the galls received had the brown interiors. Upon cutting the tissue back from the surface where the discoloration was darkest and of greatest extent to the point of least infection in the interior, it was found that the spots were lighter colored and had a water-soaked appearance. These areas were usually circular. There were rather large cavities in the badly diseased places, smaller ones in those less diseased, and very tiny ones in the lighter colored areas, which were most recently infected. Some of the light-colored pieces of tissue with cavities could be broken or cut out intact.

ISOLATIONS AND INOCULATIONS

Razor sections of the brown areas with pocketlike spaces were examined under the microscope, and motile bacteria in great numbers were found. (Pl. 1, C and D.) In the belief that this browning was a secondary infection, which had occurred in true crown-gall

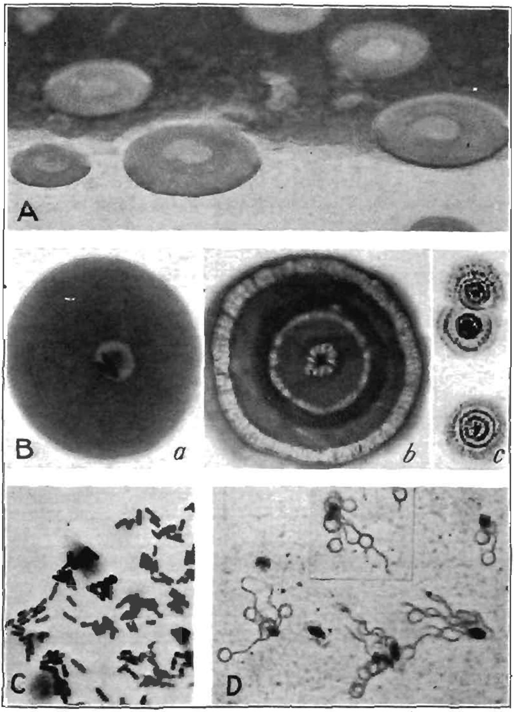
[1] Received for publication June 12, 1928; issued October, 1928. "Bacterial pocket disease" has been substituted for the term "tuberculosis" originally used because the brown pockets in the white tissue are considered the most characteristic feature of the disease.

tissue, the white and light-colored soundest tissue available was used for isolating in the hope of obtaining the crown-gall organism. Repeated platings were made from the parts of the gall which showed no discoloration. No crown-gall colony appeared, but there were numerous colonies of another type. Becoming suspicious of this constant type of colony, the writer poured plates from the parts slightly discolored and also from those of a dark color. The same type of colony (pl. 1, A) appeared throughout the different platings.

Colonies were observed in 21 to 28 hours after the plates were poured. They were very numerous on plates poured from the tube containing the macerated tissue but scattering on the dilution plates, on which they could be studied well. They were buff colored, 2 to 3 mm. in diameter, shining, mostly smooth, some irregular, but most of them round, thin with a thickened place in the center, and with rather inconspicuous concentric rings. Three days after the plates were poured, the colonies were yellow, 4 to 6 mm. in diameter, some smooth on the surface, and others rough except at the outer rim. The concentric rings were not very distinct and usually there were not more than two of them. In November, 1910, 30 young sugar beets growing in the greenhouse were inoculated with cultures from these isolations, and in a month there were ¾-inch galls on 50 per cent of them. The outgrowths were rather flat, rarely hemispherical, but frequently knobbed like the galls resulting from inoculations with the peach, hop, and daisy strains of *Bacterium tumefaciens*.

More specimens of this type of gall were received in November, 1910. The second lot came from Garden City, Kans. (fig. 1), and the third from Rocky Ford, Colo. The galls occurred on the top and sides of the crown and at various intervals on the beet down to the tip. As the tumors were fewer and smaller toward the tip, it was concluded that the infection had not begun at the root tip and worked up, but that it had worked down from the crown. Galls from both lots of beets were used for isolating, and the same buff-colored colonies, changing later to yellow, grew on the plates of both sets. There was no crown-gall colony present. Inoculations with subcultures from these two sets of plates were made on growing sugar beets (24 from the Garden City and 22 from the Rocky Ford lot). Typical tumors with the browned interiors resulted in 10 of the 24 Garden City and in 6 of the 22 Rocky Ford inoculations. The percentage of infection was never so large with this organism as with the crown-gall organism, the latter being usually almost 100 per cent in many strains. With this new gall or tubercle-forming organism, the percentages were in these two cases 41 and 27, respectively, which are high enough, however, to indicate positive results. Later it was found that when the soil was rich in nitrogenous fertilizers the percentage of galls was considerably higher.

The organism was reisolated from the Rocky Ford galls produced in the greenhouse. The yellow colonies appearing on the plates were of two types, some with smooth and others with wrinkled surfaces. In the previous isolations some of the colonies had roughened surfaces but none were distinctly wrinkled like these. The pathogenicity of the wrinkled colonies was tested by inoculating 10 sugar beets with subcultures and that of the smooth colonies by inoculating 16 sugar beets with subcultures. The wrinkled type proved as



A.—Agar plate colonies, smooth type, photographed with oblique light. × 10.
B.—Different varieties of smooth-type colonies: *a*, common growth; *b*, ringed; *c*, shadow occurring at bottom of plates; the colonies are like *b* when the agar is cut and they are able to grow to the surface. × 10.
C.—Bacteria stained in diseased tissue. × about 1,500.
D.—Flagella stained by Casares-Gil's method. × about 2,500.

infectious as the smooth one. Eight of the beets inoculated with the wrinkled type of colony and 14 of those inoculated with the smooth type became infected. These inoculations with reisolations were made in September, and the percentages of infection were high, due

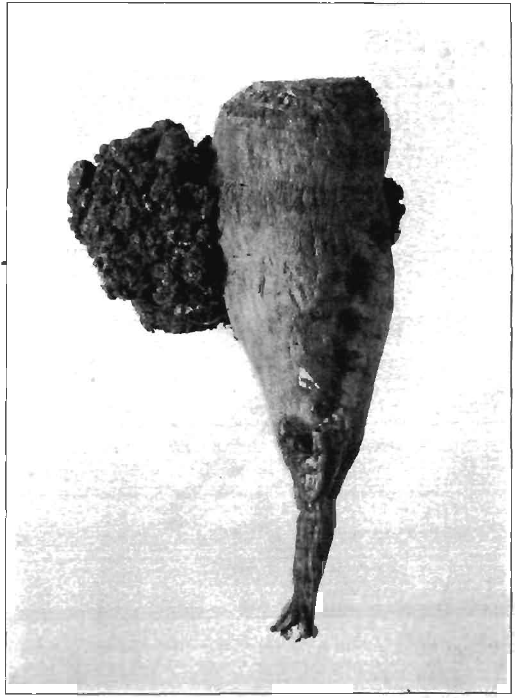


FIG. 1.—Bacterial pocket disease of sugar beet from Garden City, Kans. Photographed Nov. 7, 1910. Natural size

to good growing conditions and a better knowledge of soil requirements of the organism.

Later both wrinkled and smooth colonies grew on plates poured from a bouillon culture of a wrinkled colony. The size of the colonies varied according to the number on the plates. Those on very thinly

sown plates were often 5 to 7 mm. in diameter in three to four days. Besides these round colonies with smooth and wrinkled surfaces there was another type, yellow in color but irregular in outline. Slight marginal irregularities in colonies had been noted before but they were not so pronounced. This colony had a round center of a deep-buff color with a papillate surface, from which rootlike branches extended. Inoculations with the lobed colony proved that it was infectious also, as four of the five sugar beets inoculated with it produced the typical lesions. Plates were made from a bouillon culture of the irregular colony and on them all three types of colonies appeared, most of them being the irregular rhizoid type.

In August, 1912, sugar beets were received from Garden City, Kans., one of which had a ½-inch gall on the crown, with a smaller one adjoining it. On this beet there were six other small nodules that resembled galls, and the condition was considered a case of crown gall. Microscopic examination, however, disclosed that this was not crown gall, but the type first found in 1910. Plates were poured, and in 24 hours colonies were up on them. These were of two types, a lobed colony and a round one, but instead of being buff colored they were white, appearing bluish in transmitted light. This white color was rather disturbing, but the next day it became yellowish and three days later a decided yellow. The colonies had barely perceptible concentric rings. They were thinner than the colonies from the Colorado beets and seemed to grow more rapidly. The lobed type was not smooth on the surface like the round one.

In September, 1912, more beets with galls came from another source at Garden City, Kans. (Fig. 2.) Colonies on the plates poured from these galls were typically buff colored when first up and yellow in 24 hours. The three types—round smooth, round wrinkled, and lobed—appeared on this set of plates. Inoculations with colonies from the two Garden City isolations produced the typical galls or tubercles.

A note was published about this disease in the first bulletin dealing with the crown-gall organism.[2] It was pictured, its cultural characters were stated briefly, and it was named *Bacterium beticolum*.

Few outbreaks have been reported since 1912, and it was supposed that the disease had practically died out until November, 1923, when it appeared to a marked extent in the sugar beets grown at the Arlington Experiment Farm, Rosslyn, Va. (Fig. 3.) By a close observer or one familiar with its appearance, the disease can be noted while the beets are growing in the fields. It is usually, however, not until harvest when the beets are handled and sorted that the infection is found. As stated before, many of the tumors are of a shape and size to be mistaken for cultivation-wound calluses and often pass as such. Wound calluses, however, which consist of sound white tissue, do not carry with them the menace to sugar making that these galls do.

There were 10 full-grown sugar beets from the Arlington farm affected with the tubercular outgrowths. Some of these could be seen before the beets were pulled, as the crowns protruded a little from the ground. The soil in which these beets grew received the following treatment before the seeds were planted: A crop of winter rye

[2] SMITH, E. F., BROWN, N. A., and TOWNSEND, C. O. CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213: 194. 1911.

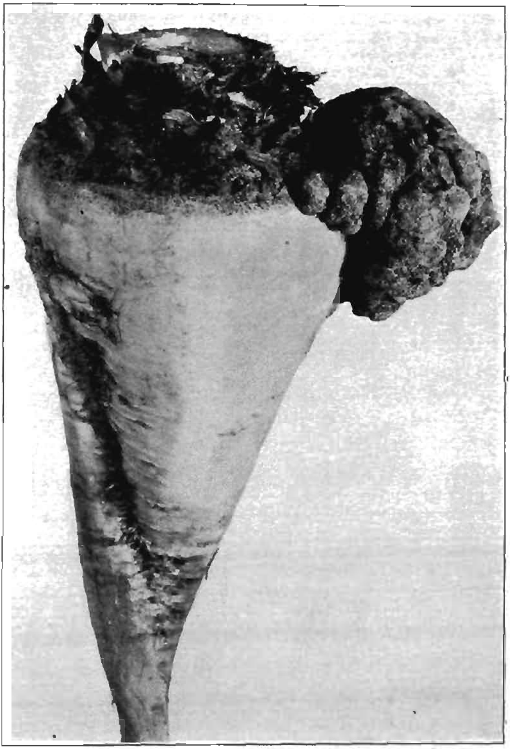


FIG. 2.—Bacterial pocket disease of sugar beet from Garden City, Kans. This type of infection can easily be mistaken for crown gall. Photographed Oct. 2, 1912. About three-fourths natural size

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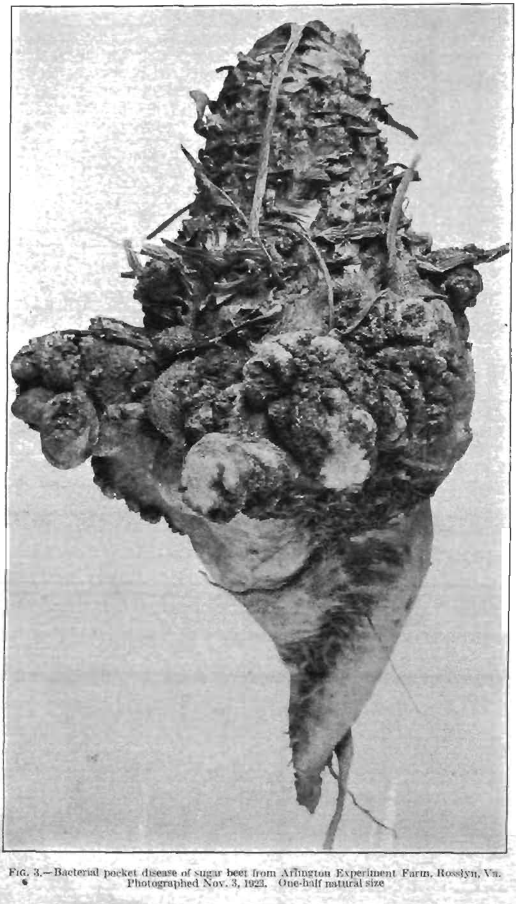

FIG. 3.—Bacterial pocket disease of sugar beet from Arlington Experiment Farm, Rosslyn, Va. Photographed Nov. 3, 1923. One-half natural size

was plowed under, and after a month had elapsed, manure was also plowed under. No other fertilizer was used, but a few weeks before planting a coating of lime was spread over the soil. Plates were made from 7 of the 10 beets, and the same yellow organism was recovered from each beet. Some of the plates contained pure cultures. The colonies were buff colored when they first appeared, as in the isolations of 1910 to 1912, and changed to yellow when older. Some had concentric rings (pl. 1, A, and B, *b*), but most of them were without rings (pl. 1, B, *a*); some had a thickened or papillate center; some were slightly irregular, but most of them were round and had a bluish margin in transmitted light. The shadow colonies were bluish (pl. 1, B, *c*) and the surface ones yellow. There were a few colonies with fish-scale markings as in the first platings of 1910. Forty-two sugar beets were inoculated with isolations from these different beets, and in one to two months 32 of them became infected with galls varying in size from 0.5 to 2 inches in diameter. When cut across all had the brown pockets (fig. 4, A) and viscid exudate. There seemed to be no difference in the infective nature of the different types of colonies, the ringed and those without rings inducing the tubercles with like facility. Reisolation plates were made from these various galls at different times, and 36 sugar beets were inoculated with the reisolation colonies. Twenty-three typical outgrowths resulted (fig. 4, B), one of which was 5 by 4 by 2 inches. The reisolation colonies were of the typical yellow color, but in one set there appeared among the yellow ones a hyaline to white colony, which in transferring was found to be rubbery. When older it became cream colored, but not yellow. Nine sugar beets were inoculated with two of these white rubbery reisolation colonies, and in two weeks four fair-sized galls with pockets were produced. (Fig. 4, C.) One of the infected beets was replanted, and two months later the outgrowth was nearly 4 inches in diameter.

Young tomato plants, Ricinus, nasturtiums, calendulas, Paris daisies, Pelargoniums, garden beets, and Bryophyllums were also inoculated with infectious cultures from the various isolations. No outgrowths occurred on any of these plants except the garden beet. The crown-gall organism, *Bacterium tumefaciens*, however, is infectious to all these hosts.

DISTINCTION BETWEEN BACTERIAL POCKET DISEASE AND CROWN GALL OF SUGAR BEETS

The common type of bacterial pocket disease as observed by the writer is one in which the outgrowths occur at the crown in nodules either singly, in groups, or coalesced. They are not always at the crown, but that is the usual position. The individual nodules are 1 to 3 cm. across and about 1 cm. thick. Frequently, however, the disease has the outward appearance of crown gall on sugar beets. (Fig. 2.) The tumor, instead of spreading over a large part of the crown in the form of nodules, occurs at one point on it, is much larger, and assumes the globose form characteristic of crown gall. In this form it can not be distinguished from crown gall except by cutting across and examining the tissue. Crown-gall tissue is white and sound. Bacterial pocket tissue is brown and has cavities which usually contain a mucilaginous substance.

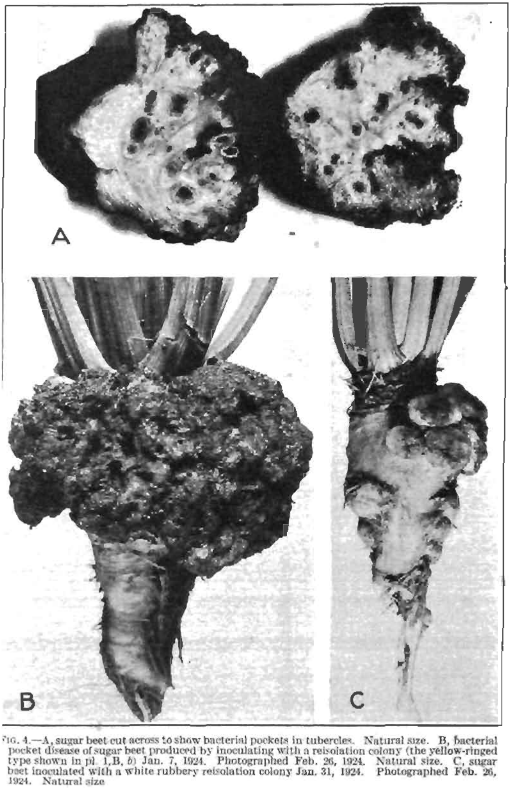

FIG. 4.—A, sugar beet cut across to show bacterial pockets in tubercles. Natural size. B, bacterial pocket disease of sugar beet produced by inoculating with a reisolation colony (the yellow-ringed type shown in pl. 1,B, *b*) Jan. 7, 1924. Photographed Feb. 26, 1924. Natural size. C, sugar beet inoculated with a white rubbery reisolation colony Jan. 31, 1924. Photographed Feb. 26, 1924. Natural size

THE CAUSAL ORGANISM

CONFUSION REGARDING ITS NAME

The organism causing the bacterial pocket disease was named *Bacterium beticolum* in 1911. In 1913 Serbinow [3] described an organism that he called *Bact. beticola*. This organism produces a so-called bacteriosis of sugar beets in which the whole root is gradually destroyed. There is no tubercle development, and the description of the organism producing the disease indicates that it is not the same as the one discussed in this paper.

Briefly, a few of the different characteristics of *Bact. beticola* Serb. are as follows:

> It appears on beef-peptone agar plates in 48 hours as a white round colony, becoming yellowish brown with a diffuse margin in three to five days; it develops gas in beef agar and gelatin when 2 per cent cane and 5 per cent grape sugar are added to them; it does not produce hydrogen sulphide or change nitrates to nitrites; its growth on potato cylinders is a muddy white, color hardly noticeable or entirely absent; it is peritrichous; it produces no capsules or chains; and neither the tissues of sugar beets infected by it nor the liquid from the tissues is slimy.

In 1915 Potebnia [4] discussed the names of both of these organisms. He stated that the name *Bacterium beticolum*, given to the bacterial pocket disease organism in 1911, is incorrect Latin and should be *Bact. beticola*. He knew that the name *Bact. beticola* had been used by Serbinow in 1913 to designate a different organism pathogenic to sugar beets, so he changed the name of the latter organism to *Bact. serbinowi* and that of *Bact. beticolum* to *Bact. beticola*. As Potebnia is right about the ending of the Latin word, this correction is accepted and the organism that produces tubercles on sugar beet should be called *Bact. beticola* (Smith, Brown, Townsend) Potebnia.

CULTURAL CHARACTERS

The beef agar, bouillon, and gelatin used in these experiments were made with beef infusion unless otherwise stated.

The colonies used in the cultural tests were all of the smooth type.

Beef-agar plates.—Colonies appear on the plates 21 to 28 hours after pouring from macerated gall tissue; they are cream colored to buff, 2 to 3 mm. in diameter, shining, mostly round and smooth (pl. 1, B, *a*), some round and wrinkled, and a few slightly lobed and either smooth or wrinkled. Many of the round smooth colonies are thin with a thickened place in the center and rather inconspicuous concentric rings. (Pl. 1, A.) In two to three days after the plates are poured, the colonies are yellow and 4 to 6 mm. in diameter, and the rings are still visible. There was an exception in the isolations from one lot of material as to initial color. The colonies came up white and were bluish in transmitted light. They became yellow in three days, however, although the shade of yellow was always light.

Beef-agar slants.—There is moderate filiform growth, flat, glistening, usually smooth, but often rugose on slants of beef agar with a pH of 6.8 to 7.1. It is yellow, translucent, viscid, and has practically no odor.

Beef bouillon.—Clouding occurs in 7 to 18 hours at a temperature of 25° C. in media with a pH range of 6.7 to 7.3. It is heavy in three to four days, a yellow ring is present, and strings of viscid growth hang down in the medium. There is also a thick growth at the bottom of the tube. Sometimes a yellow

[3] Serbinow, I. L. über die neue Bakteriose der Zuckerrübenwurzel. Zhur. "Boliezni Rastenii" 7: 237–258, illus. 1913. [In Russian. German résumé, p. 257–258.]

[4] Potebnia, A. A. [Fungous parasites of the higher plants in Kharkov and adjacent provinces.] Kharkov Prov. Agr. Expt. Sta. 1: 27–29, illus. 1915. [In Russian. Reviewed by M. Shapovalov in Phytopathology 6: [293]–295. 1916.]

pellicle, which falls immediately on handling the tube, forms. In 11 days there is a yellow color throughout the medium. The sediment in the bottom is abundant and is viscid on agitation. The odor is not unpleasant. At the optimum temperature, 29° C., growth takes place in some of the colonies in three to four hours.

Whey-agar slants.—On whey agar with a pH of 6.8 to 7.4 the organism grows luxuriantly; some colonies have a smooth surface, others are rugose.

Potato cylinders.—At first there is a light-yellow growth, which changes to a deep yellow in less than a week. Three of the colonies produce a brighter yellow than the other three. The cylinders of all are slightly darkened. The diastasic action is slight.

Blood serum.—There is a fair amount of growth on Loeffler's blood serum. Some colonies produce a smooth surface, others a papillate surface growth. The color is a deep cream in some of the colonies and yellow in others. There is no liquefaction.

Bouillon over chloroform.—There is heavy clouding in 24 hours at 23° C. in tubes of beef bouillon of pH 6.8 containing 5 c. c. of chloroform.

Fermi's solution.—The medium is clouded in 3 days and a white pellicle is formed. In 6 days the pellicle is cream colored and festoons of growth hang from it into the medium; in 13 days the pellicles of all colonies are yellow and there is a yellow precipitate, which is rather fluffy.

Uschinsky's solution.—A pellicle (incomplete with some colonies) is formed in three days. There are strings and flakes of growth in the media of all colonies. The cultures were examined again in four months just before discarding, and the media of colonies 4, 5, and 6 were found to have changed to a brown or coffee color, clear, with a heavy, lighter colored, viscid precipitate, while the media of colonies 1, 2, and 3 were decidedly lemon colored, clear, with a light-yellow viscid precipitate. Because of doubt as to colonies 4, 5, and 6, plates were poured, and pure cultures of each appeared on the plates, but none of the many subcultures therefrom browned Uschinsky in 19 days.

Cohn's solution.—There is no growth of the organism in Cohn's solution.

PHYSIOLOGICAL CHARACTERS

Liquefaction of gelatin.—In beef-gelatin plates with a pH of 7.1 kept at a temperature of 20° to 22° C., the colonies are rounded up and thicker than on beef agar. There are two types, wrinkled and smooth, but all are round. The smooth ones are ringed, but they have fewer rings than those grown on agar. The colonies even on thinly sown plates are still small (3 mm.) after four days. Liquefaction begins in six to seven days. Thickly sown plates are entirely liquefied in 14 days.

Growth in gelatin stabs of pH 6.9 at 18° to 20° C. is best at the surface but occurs along the line of puncture. Liquefaction is usually crateriform; it begins in seven to eight days and occurs slowly across the surface. All the gelatin is liquefied in 22 to 30 days.

Hydrolysis of starch.—Plates were poured with beef-infusion agar, pH 8, containing 0.2 per cent of corn starch. When the agar had hardened, smears of the organism were made across the plates. After seven days there was a fair amount of growth, and the surfaces of some of the plates were flooded with iodine solution. There was no change in the color of the medium around the streak. Tests were made with the iodine again at 14 days. At this time on either side of the streak there was an area of 8 to 10 mm., which had a clear purplish tinge with little blue flecks in it, showing that there was very little destruction of starch. Fourteen plates were treated in this way. Plates of *Bacterium phaseoli* tested for comparison gave complete starch reduction in a broad band of 3 cm. on either side of the streak.

Toleration of sodium chloride.—The organism tolerates sodium chloride up to 9 per cent. In beef bouillon (pH 7.1 titrating +11) plus 9 per cent sodium chloride all six colonies used in these tests grew, but there was no growth with 9 per cent sodium chloride when the beef bouillon titrated 0 with a pH of 8.2.

Reduction of litmus.—In three days at the surface of litmus-milk cultures there is a trace of blue that later becomes pinkish (pale vinaceous lilac [5]). Reduction of litmus is complete in 20 to 30 days. Coagulation occurs in 10 to 20 days in five of the seven colonies; the casein is digested slowly, digestion being com-

[5] Ridgway, R. Color standards and color nomenclature. 43 p., pl. XLIV. Washington, D. C., 1912.

pleted in about three months. Two infectious colonies did not coagulate milk and were slow in reducing the litmus.

REDUCTION OF METHYLENE BLUE.—All seven colonies tested decolorized methylene blue in milk in one day. Colonies 1, 2, 3, 4, and 7 coagulated it in 8 to 20 days.

REDUCTION OF NITRATES.—Nitrates are reduced to nitrites. Tests were made with nitrate-bouillon cultures 9 days old, using the starch-iodine-sulphuric acid test.

INDOL PRODUCTION.—No indol is produced. Tests were made with six colonies grown in 1 per cent peptone water for 4 and 10 days. The sodium nitrite-sulphuric acid test was used. *Bacillus coli* grown as a check in some of the same medium and tried at the same time gave a positive test.

HYDROGEN SULPHIDE PRODUCTION.—The organism produces hydrogen sulphide. The different colonies grown on potato cylinders and in beef bouillon were tested by suspending lead-acetate paper in the culture tubes. The paper blackened in two to six days.

AMMONIA PRODUCTION.—The organism produces ammonia. Both old and young cultures were tested with strips of filter paper moistened in Nessler's solution and suspended over the cultures. When the cultures were heated in a water bath, browning of the paper began immediately and reddish brown moisture ran down the sides of the tubes.

GAS PRODUCTION.—The organism is not a gas former. It was tested in fermentation tubes in the presence of the following carbon compounds: Saccharose, dextrose, lactose, maltose, glycerin, and mannit. A 1 per cent solution of each was made in a 1 per cent water solution of Difco peptone. There was no growth in the closed arm of the tube and no gas. Three colonies were tested. There was acid produced in all the solutions except that of lactose with colony 4. As some mistake was suspected, a new lactose solution was made up according to the formula used. It was inoculated and tested for pH after 18 days had elapsed, as in the first test. It was again alkaline. The pH readings are given in Table 1.

TABLE 1.—*Acid production by Bacterium beticola after a growth of 18 days in 1 per cent sugar solutions added to 1 per cent Difco peptone*

[Acidity indicated by pH readings]

Colonies	Chemicals						
	Glycerin	Dextrose	Saccharose	Lactose: First test	Lactose: Second test	Maltose	Mannit
Check	6.6	6.4	6.8	6.8	6.4	6.6	6.8
Colony 1	6.2	4.6	6.2	6.2	5.4	5.8	5.4
Colony 2	6.4	3.7	4.7	5.8	----------	5.6	6.2
Colony 4	5.4	4.4	5.2	7.1	8.0	5.4	5.3

OPTIMUM REACTION FOR GROWTH IN BEEF BOUILLON AND THE TITRABLE ACIDITY AND ALKALINITY.—The best growth in peptone beef-infusion bouillon takes place at pH 6.5 (+17), although the organism has a wide range and is not easily retarded, growing from pH 4.8 (+34) to pH 9.1 (−9). Good growth conditions are from pH 5.8 to 8.6 (+24 to −4). There is a fair amount of clouding at pH 5 (+32), only slight clouding at pH 4.8 (+34), and none at pH 4.5 (+37). Good clouding occurred at pH 8.2 to 8.6 (0 to −4), moderate at pH 9 (−8), weak at pH 9.1 (−9), and none at pH 9.5 (−13).

Tests were made to learn what changes were taking place in media of pH 9.1 and 6.6 after the organism had been growing 2, 5, 8, and 15 days. The results are shown in Table 2.

TABLE 2.—*Changes in the hydrogen-ion concentration of beef bouillon caused by the growth of Bacterium beticola in media near the optimum pH 6.6 and near the limit pH 9.1*

Number of days after inoculation	pH in bouillon of colony[a]—[Initial pH 9.1]			pH in bouillon of colony—[initial pH 6.6]		
	No. 2	No. 4	No. 6	No. 2	No. 4	No. 6
2	8.0	7.8	8.0	6.2	6.4	6.4
5	8.0	7.8	8.0	6.2	6.4	6.4
8	8.2	8.2	8.2	6.8	6.8	6.8
15	8.2	8.2	8.2	6.8	6.8	6.8

[a] At the end of 5 days the pH of the check was 8.8.

TOLERATION OF ORGANIC ACIDS.—Tests were made with citric, oxalic, and tartaric acids by adding 0.1, 0.2, and 0.3 per cent of each to neutral beef bouillon. Growth was normal in the bouillon containing 0.1 and 0.2 per cent of the acids; and clouding occurred in that containing 0.3 per cent of citric acid, but there was no growth in the bouillons containing 0.3 per cent of either oxalic or tartaric acids. With 0.4 per cent citric acid there was no growth. The pH and titrable-acidity values are shown in Table 3.

TABLE 3.—*Toleration of organic acids by Bacterium beticola*

[Acids in beef bouillon]

Citric			Oxalic			Tartaric		
pH	Fuller's scale	Character of growth	pH	Fuller's scale	Character of growth	pH	Fuller's scale	Character of growth
7.1	+11	Heavy clouding in in 20 hours.	7.1	+11	Heavy clouding in 20 hours.	7.2	+10	Heavy clouding in 20 hours.
6.1	+21	Good clouding.	5.7	+25	Good clouding.	6.2	+20	Good clouding.
5.4	+28	Do.	4.9	+33	No clouding.	5.2	+30	No clouding.
5.1	+31	No clouding in 2 weeks.						

THERMAL RELATIONS.—The organism grows at temperatures of 1.5° to 39° C. Only two out of six colonies grew at 1.5°, but all grew at 39°. None grew at 40°. The optimum temperature is about 29°.

The thermal death point is between 51° and 52° C. when beef-bouillon cultures (pH 6.8 to 7.2) are exposed in a water bath for 10 minutes. When exposure was made at 50° good growth took place. In two tests six colonies grew at 51°, but none grew at 52°. At 51.5° all colonies grew in the first test, none in the second, and only one in the third.

OXYGEN RELATIONS.—For some time it was thought that *Bacterium beticola* was a facultative anaerobe. Numerous tests were made in which beef-bouillon and beef-agar transfers of pH 6.8 were placed in jars in which the oxygen was replaced by nitrogen or in specially devised jars from which the air was exhausted. In 18 hours there would be a faint growth, but this would not continue. When a medium less favorable for growth was used, such as agar of pH 8.9 to 9.1, in which development would not begin immediately, no growth occurred. It was therefore concluded that the organism was aerobic and that its apparent facultative anaerobic tendencies were due to the quick growth of the organism before the oxygen was replaced. As stated elsewhere, growth takes place in beef bouillon of pH 6.8 in three hours at a temperature of 29° C. with some of the colonies, which were infectious in the spring of 1925.

EFFECT OF FREEZING.—Transfers were made to beef bouillon of pH 6.7 from a 1-day-old culture, and after 15 minutes plates were poured to use for colony comparisons later. The transfers were then immersed in a mixture of cracked ice and salt, where they remained frozen solid for 20 minutes. They were thawed

out quickly and more plates were poured. From 80 to 90 per cent of the colonies were killed by the freezing.

EFFECT OF DESICCATION.—The organism is somewhat resistant to drying. Sterile cover glasses receiving a drop of a 24-hour-old beef-bouillon culture were kept at room temperatures, 25° to 28.5° C. After the cultures had dried from 1 to 12 days the covers were dropped into tubes of beef bouillon. Growth took place in tubes receiving covers that had dried 7 days, but there was none in the 8 to 12 day tests. Plates were poured from the 7-day tubes and pure cultures of *Bacterium beticola* obtained.

LONGEVITY.—The organism lives for 14 months in sterile milk at room temperatures, 22° to 29° C. The milk may be dried to a jellylike consistency and the organisms remain alive. It lives from 5 to 8 months in agar and bouillon at room temperatures, and 13 to 16 months in beef bouillon if kept in the refrigerator at a temperature of 12° to 14° C.

GROWTH IN INDICATOR SUGAR AGAR.—Color changes were observed in beef agar of pH 6.7 to which 1 per cent dextrose, saccharose, galactose, lactose, and glycerin were added and the media colored with brom cresol purple, an indicator that becomes yellow in acid media. The organism grew rapidly on the sugars. The medium containing saccharose turned yellow in 48 hours without reddening; it became purple again in two weeks. The galactose and dextrose reddened in 18 hours, then turned yellow, beginning at the surface. In 42 hours the purple color returned just below the growth, and in two weeks the entire agar was purple. Lactose and glycerin became a faint greenish yellow color at the surface in 48 hours; this faint color was all through the agar in 72 hours. The next day the purple color returned to the surface, and in 7 days the lactose was purple again, but it took 15 days for the glycerin medium to become purple.

LITMUS AGARS.—The base medium consisted of 1 per cent Witte's peptone, 1 per cent agar, and litmus solution to which 1 per cent of the following sugars was added: Saccharose, dextrose, lactose, maltose, mannit, raffinose; also 0.5 per cent levulose and 0.5 per cent galactose.

In seven days there was slight reddening of the medium in saccharose, dextrose, maltose; a trace in glycerin; good reddening in mannit, galactose, and levulose, There was no reddening in raffinose or lactose. The litmus color returned after 2 days in levulose, maltose, and mannit, and in 5 to 7 days in galactose, dextrose, saccharose, and glycerin.

RELATION TO SUNLIGHT.—The organism is not very sensitive to sunlight. Thinly sown agar poured plates were exposed in bright sunlight at midday on bags of crushed ice out of doors, half of each plate being covered with black paper to serve as a check. An exposure of 65 minutes did not kill the organism, but one of 75 minutes did. One strain was able to withstand an exposure of 90 but not of 100 minutes.

COLOR PRODUCTION.—The color of the young bacterial growth is buff, but this quickly changes to a definite yellow. Yellow prevails in most media.

VIRULENCE.—Isolations made from Garden City and Rocky Ford beets in 1910 and 1912 and kept since at 12° to 15° C. and transferred several times a year were still virulent in January, 1924, after 12 and 14 years.

REACTION TO STAINS.—The organism stains readily with carbol fuchsine, gentian violet, and methyl violet. It is not acid fast and is Gram variable. This was determined by making repeated tests with the regular Gram's stain. Other Gram tests were made with 1-day agar cultures, using a mixture of carbol fuchsine, gentian violet, and buffers of different ranges (the Clark buffer series). The preparations were mordanted with Lugol's solution and decolorized with acetone. When the stain contained a buffer of high acid or high alkaline range, as pH 1 and 9, the test was positive. When the pH was about 5 and 6 the test was negative. This last test was suggested and carried out according to Stearn and Stearn's methods for testing the relation of acidity and alkalinity to Gram character,[6] and, as in their results, it seems that the hydrogen-ion concentration may play a rôle in determining the Gram character of an organism. As ordinarily tested, the organism is Gram negative.

EMENDED DESCRIPTION OF THE ORGANISM

Bacterium beticola is a short motile rod, usually paired, but it may occur singly, in clumps, or in chains of 6 to 10 elements. There are one to four flagella at either pole; these are long and in the paired rods give the appearance of peri-

[6] STEARN, E. W., and STEARN, A. E. THE CHEMICAL MECHANISM OF BACTERIAL BEHAVIOR. II. BEHAVIOR TOWARD DYES—FACTORS CONTROLLING THE GRAM REACTION. Jour. Bact. 9: 463–477, illus. 1924.

ichous flagella. The size of the rod is 0.6 to 2 μ long, 0.4 to 0.8 μ wide; irregular rms of a round or oval shape twice the ordinary size were noted. Capsules are oduced, but no spores. The organism is Gram variable; not acid fast; aerobic; lor yellow; liquefies gelatin slowly, but not Loeffler's blood serum; reduces trates; produces hydrogen sulphide and ammonia but not indol; has weak astasic action; coagulates milk; reduces litmus in 10 to 30 days, the litmus ing first blued; forms acid from dextrose, saccharose, maltose, and mannit, but ot lactose; does not produce gas; grows well in Uschinsky's and Fermi's solu-ons, making them viscid, but not at all in Cohn's; optimum temperature about)° C., maximum 39°, minimum 1.5°; thermal death point 51° to 52°; not sensi-ve to sodium chloride, tolerating 9 per cent in beef bouillon; has a pH range of 8 to 9.1 (+34 to −9); not killed readily by drying or exposure to sunlight; lives months in sterile milk at 22° to 29°; stains readily with gentian violet, methyl olet, and carbol fuchsine; pathogenic to sugar and garden beets, producing ibercles with cavities.

The index number based on the chart of the Society of American Bacteriolo-sts [7] is 502 var.–31125–1222.

NATURAL INFECTION AND CONTROL

The organism is a wound parasite, which probably gets into the igar beet through cultivation wounds or through breaks in the tiny otlets made when the beets are thinned. The disease occurs in lds heavily manured or in those that have received large quantities sodium or potassium nitrate. Up to the present it has not been iown to occur under other conditions. The control therefore is ther easy and obvious.

SUMMARY

A gall disease of sugar beets in the nature of tubercle outgrowths th pockets is described in this paper. It is produced by an organism which the name *Bacterium beticola* has been given (corrected from *ict. beticolum*). The organism is a wound parasite, which stimu-les the tubercles to form, discolors the gall tissue, and produces vities, which usually contain a viscid fluid. The tubercles with ckets and exudate were reproduced on sugar beets and garden ets by inoculation.

In outward appearance the disease frequently resembled crown ll but can easily be distinguished from it by cutting through the tgrowth and noting whether or not there are pockets and stained sue within. In crown gall the tissue is white and sound.

The disease so far as known occurs only in soil rich in nitrogenous tilizers, and on this account its control is not difficult. It is own to occur in Kansas, Colorado, and Virginia.

The parasite is a yellow, polar flagellate, and is not known to oduce tubercles on any other plants than sugar and garden beets. ie index number is 502 var.–31125–1222.

[7] Society of American Bacteriologists. Descriptive chart. [2] p. [1924.

GROWTH AND SENESCENCE IN RED DANISH COWS AS MEASURED BY THE RATE OF MILK SECRETION[1]

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INTRODUCTION

A recent paper by Davidson (*5*)[2] introduces a new form of growth and senescence equation, which he has developed as a result of an analysis of certain records of live weight and milk yield of purebred Jersey cows. Davidson's equation is of the form

$$\log Y = a - be^{-kt} - ce^{ht} \qquad (1)$$

in which Y is the rate of milk yield at the time or age t. The derivation of the equation is fully explained in the reference given. It is sufficient here to say that k represents the rate of decrease in growth power of the body cells, while h represents the rate of loss in physiological activity of the cells. The constants b and c are said to locate the curve in time; that is, their values depend upon the time origin but are independent of the time unit. The values of k and h vary directly with the time unit but are independent of the time origin.

The value of any equation of this kind can be fully developed only by its application to observed facts. As mentioned, Davidson has applied it successfully to certain records of the Jersey breed. It is applied in the present paper to somewhat similar milk-yield data of the Red Danish breed.

While t in the equation may be measured in any unit from any origin, it is suggested in the interest of uniformity, that t be measured in years from birth, being thus a direct measure of age in years. It seems desirable also to measure milk yield on an energy basis in order to have directly comparable values so far as variability in composition of the milk is concerned. This basis of measuring milk yield was suggested by Gaines and Davidson (*8*) and has recently been more fully elaborated (*7*).

SOURCE AND TREATMENT OF DATA

The cooperating Danish agricultural societies (*12*) publish a herdbook of cows of the Red Danish dairy breed, to which are admitted cows of that breed which meet certain requirements. The essential feature of the entrance requirements pertains to the milk production of the cow. It is required that the cow shall produce at least 160 kgm. of "butter" (about 315 pounds of fat) as an average per year for at least three years, and shall have an average fat percentage of not less than 3.60. However, cows having an average fat percentage of 3.45 to 3.60 may be admitted on the same general terms, except that the average yield shall be not less than 175 kgm. of "butter" (about 345

[1] Received for publication May 28, 1928; issued October, 1928.
[2] Reference is made by number (italic) to "Literature cited," p. 180.

pounds of fat). In some respects this system is like the American system of advanced registry, but since the entrance requirements are based on the yield over a period of years there is no object of prolonging lactation by delayed breeding and the cows are bred to freshen every year. It should be noted that this breeding feature is in line with economical production (*6, 13*) and seems to be a requirement that might well be emulated by advanced registries in this country.

The "butter" yield is computed from the weight of milk and its percentage fat content by the formula $B = \frac{M(f-0.15)}{86}$ where B is butter and M is milk, both in kilograms, and f is fat percentage.

The herdbooks include, in addition to the yearly records of the cow herself, also the yearly records of her female ancestors so far as available. The yearly records are for the fiscal year beginning October 1, and include the milk and butter yields. Any fiscal-year record which is considered by the authorities in charge of the herdbooks to be not representative is inclosed in brackets and excluded from the average. The published records include also the date of birth of the animals.

The data of the present paper are taken from the records of the ancestors of the herdbook cows entered in volumes 1, 2, and 3. The records of the herdbook cows themselves are not used for the reason that the entrance requirements must exclude a certain proportion of the population. This selective effect would apply also to their ancestors, but in a much smaller degree. The records of all ancestors so far as reported have been used, but bracketed yearly records have been excluded.

The milk and butter records were extracted in four main groups according to the age of the cow in years at a date 1.5 months following the beginning of the fiscal year, viz, Group 1, 1 to 1.25, 2 to 2.25, etc.; Group 2, 1.25 to 1.5, 2.25 to 2.5, etc.; Group 3, 1.5 to 1.75, 2.5 to 2.75, etc.; Group 4, 1.75 to 2, 2.75 to 3, etc. The total milk and butter yield of each age class was then computed by direct summation of the yearly records excluding bracketed records. From these totals were computed the average fat percentages and energy yields by use of the formulas, $f = 86B/M + 0.15$, where f is the average fat percentage; and $F.\ C.\ M. = \frac{0.9314\,M + 28.439B}{N}$, where N is the number of records, M is milk in kilograms, B is butter in kilograms, and *F. C. M.* is energy value in terms of pounds of 4 per cent milk. The *F. C. M.* estimate is the equivalent of the usual $0.4M + 15F$ formula (*8*), converting to the English unit. The only excuse for conversion to the English unit is to make the records directly comparable with records in this country so far as the unit of weight is concerned. The results of the above computations are shown graphically in Figure 1.

The frequency distributions of Figure 1 show an orderly irregularity and seem to mean that there is a pronounced tendency for the Danish dairyman to have his cows freshen during September and October.

The fat percentage curve for the most part lies between 3.7 and 3.8, and evidences a tendency to decrease very slightly with age. As above noted, certain requirements are enforced with reference to the fat percentage of the herdbook cows and these requirements are evidently rather rigorous. In the introduction to volume 1 is given

a summary (*12, Bd. 1, p. XXI, Table 10*) which shows the average fat percentage of all cows tested as 3.36 in 1900, and this has gradually increased to 3.66 in 1920. It is evident, therefore, that the herdbook requirement as to fat percentage selects not only the higher testing individuals, but also higher testing mothers, all of which may be regarded as evidence that the fat percentage character is heritable and subject to modification by selective mating.

The *F. C. M.* or energy yield curve is the one of principal interest from the point of view of this paper. It shows the familiar, rapidly rising values up to 6 years, fairly stationary values from 6 to 11 years, with a descending tendency after that age. The work is next to fit equation (1) to these energy yield data of Figure 1.

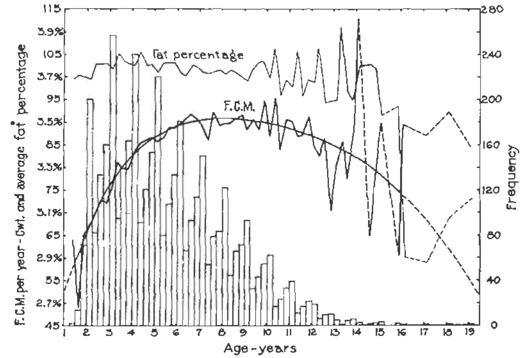

FIG. 1.—Relation of age to energy yield in Red Danish cows. Age is computed to a date 1.5 months following the beginning of the fiscal year. The energy yield is for the fiscal year and is expressed in terms of fat-corrected milk (*F. C. M.*) or 4 per cent milk. One pound *F. C. M.*=340 large calories. The smooth curve is that of Figure 2. The fat percentage shown by the upper lighter line curve is a weighted average. The numbers of records at the age classes are shown by the columns. There are 4,109 annual records of 740 different cows, an average of 5.55 years for each cow

FITTING THE EQUATION

In the first place, it seems desirable to try to eliminate some of the sharp irregularities of the *F. C. M.* curve of Figure 1 by the use of a longer age interval, say 6 or 12 months. The groups of Figure 1 have been, accordingly, combined into a new grouping, viz, 1.5 years (1.25 to 1.75), 2.0 years (1.75 to 2.25), ... 5.5 years (5.25 to 5.75), 6.0 years (5.5 to 6.5), 7.0 years (6.5 to 7.5), ... The new grouping involves a duplication of the class at 5.5 to 5.75 (107 records), which group now appears in both the 5.5 and 6.0 year classes. These new age values, 1.5, 2.0, 2.5, etc., are to constitute the values of t in equation (1).

The corresponding mean *F. C. M.* values are shown numerically in Table 1, column Y, and graphically in Figure 2. From them are to be derived the constants of equation (1). It will be noted that the

last three observations of Figure 2 are out of line with the trend of the other values. These observations are for one and the same cow (mother's mother's mother of No. 1481), and since they seem to represent a very unusual animal they are excluded in fitting the equation.

For the purpose of fitting equation (1) to the data of Table 1 it is convenient to write it,

$$y = a - be^{-kx} - ce^{hx} \tag{2}$$

in which $y = \log Y$ and x is time in units of six months with origin at the first observation, $x = 2t - 3$. The constants of equation (2) may be readily transformed to those of equation (1). The equation is not

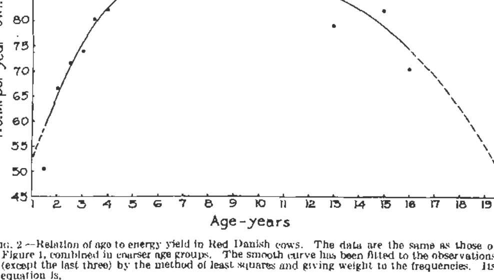

FIG. 2.—Relation of age to energy yield in Red Danish cows. The data are the same as those of Figure 1, combined in coarser age groups. The smooth curve has been fitted to the observations (except the last three) by the method of least squares and giving weight to the frequencies. Its equation is,

$$\log F. C. M. \text{ (pounds)} = 3.977502 - 0.437979e^{[illegible]} - 0.00156870e^{[illegible]}$$

in which t is age in years, origin at birth, and reckoned to a date 5.5 months following the beginning of the fiscal year

adapted to the application of direct algebraic methods. A first approximation of the constants may be made by graphic methods and these values then corrected by the method of least squares.

For the first-approximation constants the same symbols may be used in the upper case, $y_1 = A - Be^{-Kx} - Ce^{Hx}$. In data of the present nature the last term may be neglected at low values of x, and the second term may be neglected at high values of x. For low values of x we may write $A - y = Be^{-Kx}$ or $\log (A - y) = \log B - 0.4343Kx$. Likewise, for high values of x, $\log (A - y) = \log C + 0.4343Hx$. It is not difficult to guess at a value for A which is near its rightful value. From Figure 2 one may guess that if the left-hand portion of the curve were continued without the depressing influence of the last term, it would reach a value of 9,400 to 9,500, say 9,450 pounds. The logarithm of 9,450 is 3.975 and we may assume $A = 3.975$.

TABLE 1.—*Age changes in the rate of milk secretion of Red Danish Cows*[a]

t	x	n	f	Y	y	y_1	y_2	Y_2	ΔY
1.5	0	16	3.70	5,060	3.70415	3.75408	3.76988	5,887	−827
2.0	1	271	3.69	6,654	3.82308	3.80241	3.81537	6,537	+117
2.5	2	215	3.76	7,163	3.85509	3.83980	3.85059	7,089	+74
3.0	3	417	3.74	7,409	3.86976	3.86895	3.87782	7,548	−139
3.5	4	233	3.78	8,009	3.90358	3.89141	3.89881	7,922	+87
4.0	5	403	3.75	8,189	3.91323	3.90872	3.91491	8,221	−32
4.5	6	211	3.78	8,569	3.93293	3.92201	3.92727	8,458	+111
5.0	7	373	3.73	8,607	3.93485	3.93213	3.93661	8,642	−35
5.5	8	187	3.76	8,787	3.94384	3.93978	3.94361	8,782	+5
6.0	9	494	3.73	8,931	3.95090	3.94541	3.94875	8,887	+44
7.0	11	410	3.73	8,975	3.95303	3.95229	3.95481	9,012	−37
8.0	13	327	3.72	9,035	3.95593	3.95492	3.95694	9,056	−21
9.0	15	264	3.70	9,011	3.95477	3.95448	3.95609	9,038	−27
10.0	17	176	3.75	9,050	3.95665	3.95153	3.95288	8,972	+78
11.0	19	106	3.67	8,856	3.94724	3.94621	3.94742	8,860	−4
12.0	21	63	3.67	8,726	3.94082	3.93831	3.93954	8,700	+26
13.0	23	25	3.60	7,876	3.89631	3.92736	3.92881	8,488	−612
14.0	25	13	3.64	8,750	3.94201	3.91258	3.91458	8,215	+535
15.0	27	6	3.62	8,180	3.91275	3.89286	3.89589	7,868	+312
16.0	29	3	3.27	7,020	3.84634	3.86672	3.87146	7,438	−418
17.0	.....	1	2.88	8,727	.....	.....	3.83962	6,912	.....
18.0	.....	1	3.08	9,210	.....	.....	3.79815	6,283	.....
19.0	.....	1	3.16	8,479	.....	.....	3.74417	5,548	.....

[a] t = Age in years, origin at birth.
$x = 2t - 3$ = Age in units of 6 months, origin at $t = 1.5$.
n = Number of records.
f = Average fat percentage.
Y = Average energy yield in terms of pounds of 4-per-cent milk.
$y = \log_{10}$ of Y.
y_1 = Calculated y by first-approximation equation.
y_2 = Calculated y by second-approximation equation.
Y_2 = Calculated energy yield by second-approximation equation, pounds of 4-per-cent milk.
$\Delta Y = Y - Y_2$ = Observed minus calculated energy yield, pounds of 4-per-cent milk.

The next step is to plot log $(A-y)$ against x. This is done in Figure 3 and the two straight lines are drawn to fit the plotted values as well as may be by visual inspection. The K line intercepts the y axis at $\bar{1}.34$ and this is log B, consequently $B = 0.219$. It cuts the ordinate at $x = 10$ at $\bar{2}.25$, that is, $-0.4343K = \frac{\bar{2}.25 - \bar{1}.34}{10}$, from which $K = 0.251$. If A is chosen grossly too large or too small this may be detected in a systematic curvature of the plot values. This is the test used by Brody (*1, Fig. 3*), but it is not a very sensitive test.

The observations at the higher values of x, Figure 3, are rather equivocal for determination of the H line. The line drawn cuts the ordinate at $x = 16$ at $\bar{2}.25$, and at $x = 28$, at $\bar{2}.975$. Consequently, $0.4343H = \frac{\bar{2}.975 - \bar{2}.25}{28 - 16}$, and $H = 0.139$. If this line is projected to the left it intercepts the y axis at $\bar{2}.25 - 16\ \frac{\bar{2}.975 - \bar{2}.25}{12} = \bar{3}.283$. That is, log $C = \bar{3}.283$, and $C = 0.00192$.

We have therefore as a first approximation, $y_1 = 3.975 - 0.219e^{-0.251x} - 0.00192e^{0.139x}$. The values calculated from this equation are shown in the seventh column of Table 1. These values are derived at once by the use of a computing machine by negative summation of the products $B \cdot 2$ and $C \cdot 3$ with A, where 2 and 3 refer to the values in these columns of Table 2. The differences, Δy_1, are given in the last column of Table 2. They are the observed values minus the calculated

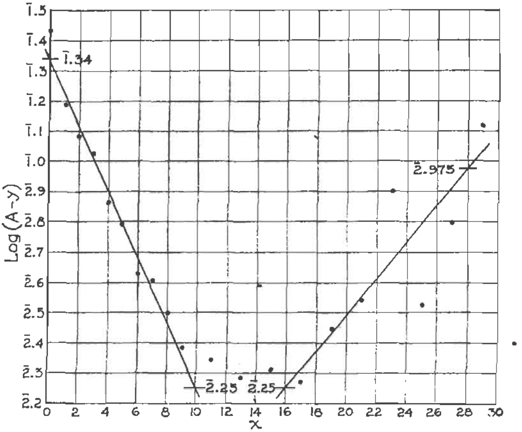

FIG. 3.—Graphic determination of the first-approximation constants

The next step is to derive corrections to the approximate constants, which we may designate ΔA, ΔB, etc., such that $(A+\Delta A)=a$, $(B+\Delta B)=b$, etc., where a, b, etc., are the correct values by the least-squares criterion. The details of the calculus of this process are given in various texts (*11*, *Chap. VI*). This leads to a new series of 20 observation equations of the form

$$\Delta A - e^{-Kx}\Delta B - e^{Hx}\Delta C + Bxe^{-Kx}\Delta K - Cxe^{Hx}\Delta H = \Delta y_1$$

The normal equations of these new observation equations are as follows:

$$+(\Sigma n)\Delta A - (\Sigma ne^{-Kx})\Delta B - (\Sigma ne^{Hx})\Delta C + B(\Sigma nxe^{-Kx})\Delta K - C(\Sigma nxe^{Hx})\Delta H = +\Sigma n\Delta y_1$$

$$-(\Sigma ne^{-Kx})\Delta A + [\Sigma n(e^{-Kx})^2]\Delta B + (\Sigma ne^{-Kx}e^{Hx})\Delta C - B[\Sigma nx(e^{-Kx})^2]\Delta K + C(\Sigma nxe^{-Kx}e^{Hx})\Delta H = -\Sigma ne^{-Kx}\Delta y_1.$$

$$-(\Sigma ne^{Hx})\Delta A + (\Sigma ne^{-Kx}e^{Hx})\Delta B + [\Sigma n(e^{Hx})^2]\Delta C - B(\Sigma nxe^{-Kx}e^{Hx})\Delta K + C[\Sigma nx(e^{Hx})^2]\Delta H = -\Sigma ne^{Hx}\Delta y_1.$$

$$+B(\Sigma nxe^{-Kx})\Delta A - B[\Sigma nx(e^{-Kx})^2]\Delta B - B(\Sigma nxe^{-Kx}e^{Hx})\Delta C + B^2[\Sigma nx^2(e^{-Kx2})]\Delta K - BC(\Sigma nx^2e^{-Kx}e^{Hx})\Delta H = +B\Sigma nxe^{-Kx}\Delta y_1.$$

$$-C(\Sigma nxe^{Hx})\Delta A + C(\Sigma nxe^{-Kx}e^{Hx})\Delta B + C[\Sigma nx(e^{Hx})^2]\Delta C - BC(\Sigma nx^2e^{-Kx}e^{Hx})\Delta K + C^2[\Sigma nx^2(e^{Hx})^2]\Delta H = -C\Sigma nxe^{Hx}\Delta y_1$$

The factor n is introduced in the normal equations to give weight to the number of records represented at each value of x. This is equivalent to weighting or multiplying each observation equation by the square root of its n. Since the standard error of the means repre-

sented by each of the observation equations should vary inversely as $\sqrt{n}$ this seems to be a fair method of weighting.

For the computation of the numerical coefficients in the normal equations the work may be arranged as in Table 2. The values in column 2 of Table 2 may be obtained by inserting the constant factor $e^{-0.251}=0.7780224$ in the computing machine and multiplying successively by the last obtained value as recorded. At $x=9$ the factor is changed to $e^{-0.502}=0.6053188$. The final result at $x=29$ is independently computed by the use of logarithms, and agreement of the results checks the entire column. A similar procedure is followed for column 3. All of the coefficients are obtainable directly from Table 2 either by summation of the columns or by summation of the products of pairs of columns, by the use of a computing machine. If it is not desired to take account of the differences in the number of records contributing to the mean observations, columns 6, 7, 8, 9, and 10 of Table 2 will be omitted, thus considerably reducing the work of computation. That is to say, the values of n in column 6 are taken as unity throughout, and consequently the values of columns 7, 8, 9, and 10 become identical with those of columns 2, 3, 4, and 5, respectively. Where the observed mean values are fairly regular, this shortened procedure may be fully justified.

TABLE 2.—*Data for computation of numerical coefficients of the normal equations*

x	2 e^{-Kx}	3 e^{Hx}	4 xe^{-Kx}	5 xe^{Hx}	6 n	7 ne^{-Kx}	8 ne^{Hx}	9 nxe^{-Kx}	10 nxe^{Hx}	11 Δy_1
0	1.00000	1.0000	0	0	16	16.00000	16.0000	0	0	−0.04993
1	.77802	1.1491	.77802	1.1491	271	210.84342	311.4061	210.84342	311.4061	+.02067
2	.60532	1.3205	1.21064	2.6410	215	130.14380	283.9075	260.28760	567.8150	+.01519
3	.47095	1.5174	1.41285	4.5522	417	196.38615	632.7558	589.15845	1,898.2674	+.00081
4	.36641	1.7437	1.46564	6.9748	233	85.37353	406.2821	341.49412	1,625.1284	+.01217
5	.28508	2.0037	1.42540	10.0185	403	114.88724	807.4911	574.43620	4,037.4555	+.00451
6	.22180	2.3025	1.33080	13.8150	211	46.79980	485.8275	280.79880	2,914.9650	+.01092
7	.17257	2.6459	1.20799	18.5213	373	64.36861	986.9207	450.58027	6,908.4449	+.00272
8	.13426	3.0405	1.07408	24.3240	187	25.10662	568.5735	200.85296	4,548.5880	+.00408
9	.10446	3.4939	.94014	31.4451	494	51.60324	1,725.9866	464.42916	15,533.8794	+.00549
11	.06323	4.6136	.69553	50.7496	410	25.92430	1,891.5760	285.16730	20,807.3360	+.00074
13	.03827	6.0922	.49751	79.1986	327	12.51429	1,992.1494	162.68577	25,897.9422	+.00101
15	.02317	8.0447	.34755	120.6705	264	6.11688	2,123.8008	91.75320	31,857.0120	+.00029
17	.01403	10.6229	.23851	180.5893	176	2.46928	1,869.6304	41.97776	31,783.7168	+.00512
19	.00849	14.0274	.16131	266.5206	106	.89994	1,486.9044	17.09886	28,251.1836	+.00103
21	.00514	18.5230	.10794	388.9830	63	.32382	1,166.9490	6.80022	24,505.9290	+.00251
23	.00311	24.4594	.07153	562.5662	25	.07775	611.4850	1.78825	14,064.1550	−.03105
25	.00188	32.2983	.04700	807.4575	13	.02444	419.8779	.61100	10,496.9475	+.02943
27	.00114	42.6495	.03078	1,151.5365	6	.00684	255.8970	.18468	6,909.2190	+.01989
29	.00069	56.3181	.02001	1,633.2249	3	.00207	168.9543	.06003	4,899.6747	−.02098

The following checks assure the accuracy of the arithmetic up to this point except as to column 11: $\Sigma 2\cdot 8=\Sigma 3\cdot 7=1934.904069067$; $\Sigma 4\cdot 8=\Sigma 5\cdot 7=11{,}229.890843528$; $\Sigma 5\cdot 8-\Sigma 3\cdot 10=3{,}059{,}557.16058729$; $\Sigma 5\cdot 9=\Sigma 4\cdot 10=98{,}484.338274958$.

Column 11 is derived from Table 1, $\Delta y_1=y-y_1$

The normal equations in terms of Table 2 are:

ΔA	ΔB	ΔC	ΔK	ΔH	Absolute terms
$+\Sigma 6$	$-\Sigma 2\cdot 6-$	$\Sigma 3\cdot 6+$	$B\Sigma 4\cdot 6-$	$C\Sigma 5\cdot 6$	$=+\Sigma 6\cdot 11$
$-\Sigma 2\cdot 6+$	$\Sigma 2\cdot 7+$	$\Sigma 3\cdot 7-$	$B\Sigma 4\cdot 7+$	$C\Sigma 5\cdot 7$	$=-\Sigma 7\cdot 11$
$-\Sigma 3\cdot 6+$	$\Sigma 3\cdot 7+$	$\Sigma 3\cdot 8-$	$B\Sigma 4\cdot 8+$	$C\Sigma 5\cdot 8$	$=-\Sigma 8\cdot 11$
$+B\Sigma 4\cdot 6-$	$B\Sigma 4\cdot 7-$	$B\Sigma 4\cdot 8+$	$B^2\Sigma 4\cdot 9-$	$BC\Sigma 5\cdot 9$	$=+B\Sigma 9\cdot 11$
$-C\Sigma 5\cdot 6+$	$C\Sigma 5\cdot 7+$	$C\Sigma 5\cdot 8-$	$BC\Sigma 5\cdot 9+$	$C^2\Sigma 5\cdot 10$	$=-C\Sigma 10\cdot 11$

From Table 2:

$$
\begin{array}{lllll}
\Sigma 6 & = 4,213. & & & \\
\Sigma 2\cdot 6 & = 989.87202 & & & \\
\Sigma 3\cdot 6 & = 18,212.3751 & & & \\
\Sigma 4\cdot 6 & = 3,981.00805 & \text{and} & B\Sigma 4\cdot 6 & = 871.84077 \\
\Sigma 5\cdot 6 & = 237,819.0655 & \text{and} & C\Sigma 5\cdot 6 & = 456.61261 \\
\Sigma 6\cdot 11 & = 21.39635 & & & \\
\Sigma 2\cdot 7 & = 447.89453 & & & \\
\Sigma 3\cdot 7 & = 1,934.9041 & & & \\
\Sigma 4\cdot 7 & = 1,130.62666 & \text{and} & B\Sigma 4\cdot 7 & = 247.60725 \\
\Sigma 5\cdot 7 & = 11,229.8908 & \text{and} & C\Sigma 5\cdot 7 & = 21.56139 \\
\Sigma 7\cdot 11 & = 8.3705999 & & & \\
\Sigma 3\cdot 8 & = 164,753.91 & & & \\
\Sigma 4\cdot 8 & = 11,229.8908 & \text{and} & B\Sigma 4\cdot 8 & = 2,459.3461 \\
\Sigma 5\cdot 8 & = 3,059,557.2 & \text{and} & C\Sigma 5\cdot 8 & = 5,874.3498 \\
\Sigma 8\cdot 11 & = 51.912017 & & & \\
\Sigma 4\cdot 9 & = 4,526.0373 & \text{and} & B^2\Sigma 4\cdot 9 & = 217.07327 \\
\Sigma 5\cdot 9 & = 98,484.338 & \text{and} & BC\Sigma 5\cdot 9 & = 41.410694 \\
\Sigma 9\cdot 11 & = 23.8133664 & \text{and} & B\Sigma 9\cdot 11 & = 5.2151272 \\
\Sigma 5\cdot 10 & = 62,928.914. & \text{and} & C^2\Sigma 5\cdot 10 & = 231.98115 \\
\Sigma 10\cdot 11 & = 422.98409 & \text{and} & C\Sigma 10\cdot 11 & = .8121294
\end{array}
$$

And hence the normal equations are:

$$
\begin{array}{l}
+4213.\ \Delta A - 989.87202\Delta B - 18,212.3751\Delta C + 871.84077\,\Delta K \\
\qquad - 456.61261\Delta H = +21.39635 \\
-989.87202\ \Delta A + 447.89453\Delta B + 1,934.9041\Delta C - 247.60725\,\Delta K \\
\qquad + 21.56139\Delta H = -8.3705999 \\
-18,212.3751\Delta A + 1,934.9041\Delta B + 164,753.91\Delta C - 2,459.3461\Delta K \\
\qquad + 5,874.3498\Delta H = -51..912017 \\
+871.84077\ \Delta A - 247\ 60725\Delta B - 2,459.3461\ \Delta C + 217.07327\,\Delta K \\
\qquad - 41.410694\Delta H = +5.2151272 \\
-456.61261\ \Delta A + 21.56139\ \Delta B + 5,874.3498\ \Delta C - 41.410694\,\Delta K \\
\qquad + 231.98115\Delta H = -0.8121294
\end{array}
$$

By solution of these equations we have:

$$
\begin{array}{l}
\Delta A = +0.002502 \\
\Delta B = -0.013702 \\
\Delta C = +0.00040833 \\
\Delta K = +0.001570 \\
\Delta H = -0.007363
\end{array}
$$

and applying these corrections to the first-approximation constants, remembering that $a = (A + \Delta A)$, $b = (B + \Delta B)$, etc., we have the second-approximation equation:

$$y_2 = 3.977502 - 0.205298e^{-0.252570x} - 0.00232833e^{0.131637x}$$

In order to convert this to read reckoning time in years with origin at birth, K and H are multiplied by 2, while $b = 0.205298/e^{-3(0.262570)} = 0.437979$ and $c = 0.00232833/e^{3(0.131637)} = 0.00156870.$

The final equation is therefore:

$$\log F.\ C.\ M. = 3.977502 - 0.437979e^{-0.505140t} - 0.00156870e^{0.263274t}$$

the curve of which is shown in Figures 1 and 2. The equation reads in pounds. To make it read in kilograms it is necessary merely to subtract 0.343337 from the a constant (1 pound = 0.45359 kgm. and $\log 0.45359 = -0.343337$).

DISCUSSION

The measurement of growth and senescence by the quantity of milk yielded during various years of the cow's life may be regarded as a measurement of vital activity at the corresponding ages. Since milk secretion is very profoundly connected with reproduction and in the natural course of life is manifest periodically for a limited time following parturition, there are obvious grounds for measuring age of the cow at the date of calving, and for measuring the rate of milk secretion at its flush a few days after calving. From this point of view, the seven-day records of the Holstein breed have much merit.

There is also good reason for measuring the rate of milk secretion by the yield over a longer period, since we then consider both the rate of yield at the start of lactation and the rate of decline with advance in lactation. The yearly yield is a composite measurement, and clearly any thorough analysis of the problem should consider separately the two items, initial rate of yield and rate of decrease in rate of yield.

The above considerations are not completely satisfied by the present data. They deal with successive 12-months periods without regard to the position of the point at which calving occurs in the periods, and age is computed to a date 1.5 months following the beginning of the period.

A point of interest in connection with the fitted curve of equation (1) is the age of maximum yield. From the first derivative of the equation, the curve reaches a maximum when $t = \frac{\log \frac{bk}{ch}}{0.4343(k+h)}$, the constants being taken as positive values. From the final values of the constants the maximum is reached when $t = 8.18$. By computing age to the middle of the periods, the maximum is reached at 8.6 year of age.

From either Figure 1 or Figure 2 it is apparent that equation (1) as fitted conforms very well to the trend of the observations. The observation at 1.5 years, however, is quite far below the calculated value. On turning back to the 16 original records from which this observation is derived, it was found that several of them were for less than 250 days. It is quite probable that this observation does not fully represent the yield to be expected of cows of this age because of the inclusion of heifers which calved for the first time so late in the fiscal year as to make the time in milk abnormally short.

The various types of equations that have been used by different investigators to express the relation between age and milk yield are of interest. Some of them are:

	Author	Equation
(I)	Pearl (*9*)	$y = a + bx + cx^2 + d \log x$
(II)	Clark (*4*)	$y = a + bx + cx^2 + dx^3$
(III)	Sanders (*14*)	$\log y = a + bx + cx^2 + dx^3$
(IV)	Brody et al. (*3*)	$y = ae^{-kx} - be^{-hx}$
(V)	Brody (*2*)	$y = a - be^{-kx}$
(VI)	Davidson [3]	$y = a - be^{-kx} - ce^{hx}$
(VII)	Davidson (*5*)	$\log y = a - be^{-kx} - ce^{hx}$

The "logarithmic" equation of Pearl was the first of these to be put forward, and it has been used quite extensively. There is a good

[3] Unpublished. This is an obvious modification of (V). That is, the modification is obvious enough after Davidson's work.

deal of enchantment in the very word "logarithmic" and the equation is an adaptable one, *provided the x origin is shrewdly chosen*. The log term disappears entirely at $x=1$; and at $x=0$ it follows that $y=-\infty$. At very low values of x the log term changes very rapidly with change in x.

It has been pointed out by Brody (*2*) and Davidson that Pearl's equation is a purely empirical one.

The equations of Clark and Sanders seem to belong also in the class of empirical equations. Their constants are not susceptible of any interpretation of physical significance.

The equation (IV) of Brody et al. was introduced to test the possibility of interpreting the change in milk yield with age as a function of some chemical reaction, the general course of which has been determined by the physical chemist. The constants k and h are velocity constants. The equation was fitted successfully to seven-day Holstein records but did not seem to conform very well with the observed values for yearly yields. Just why the equation should apply in the case of seven-day records and not in the case of longer-time records is not clear.

Brody has later applied his growth equation (V), $y=a-be^{-kx}$, to a wide assortment of age and milk-yield data, up to the age of maximum production. This equation, of course, does not apply to the decreasing rates of yield which come at advanced ages.

Equation (VII) is that of Davidson, which is used in the present paper. Davidson has employed (but not published) a modification of Brody's equation. This modified form is given as (VI) in the above list. It will be noted that (VI) bears the same relation to (VII) as (II) bears to (III).

Davidson (*5*) has used a growth equation which bears a similar definite relation to Brody's, viz., $\log y=a-be^{-kx}$, where y is the growth or weight attained by the animal.[4] The difference between these two equations is interesting. Both are intended to apply to growth only after the *rate* of growth has reached its maximum. After this time, according to Brody's equation, the rate of growth decreases at a rate which is a constant proportion (k) of the rate of growth of the *whole organism*. According to Davidson's equation the rate of growth decreases at a rate which is a constant proportion (k) of the rate of growth of the *average cell* of the organism (assuming the number of cells proportional to the weight of the animal). The k's in the two equations have therefore somewhat different meanings. Brody has applied his growth equation successfully to the age changes in weight of Jersey cows (as well as a great variety of other growth data). Davidson has applied his growth equation successfully to the same data of growth in weight of Jersey cows.

This is an interesting illustration of the often observed fact that the same data may lend themselves to two rather different postulates. If one is to use the conformity of the two equations to the observed values as evidence of the support which they afford to the two postulates, respectively, it is quite evident that some precise method of fitting the equations must be followed. Although it is somewhat apart from the subject of this paper, it has seemed of

[4] This equation was suggested by Wright (*19*), in the form $\log \log \frac{k}{y}=a(b-x)$.

interest to compare the growth equations of Brody and Davidson when fitted by exact methods. For this purpose the writers have used Davidson's (*5*) Table 7 giving the change in weight with age of original entry Jersey cows. The equations have been fitted by least squares without regard to frequencies. The method will be apparent from the discussion of equation (1). It is necessary to tabulate only values corresponding to those of columns 1, 2, and 4 of Table 2. The fitted equations and the sum of the squared deviations are as follows:

Equations	$\Sigma\Delta^2$
$y = 953.93 - 277.73e^{-0.512408t}$	745.44
$\log y = 2.979189 - 0.248057e^{-0.547614t}$	748.02

In these equations t is time in years with origin at birth.

As judged by the sum of the squared deviations, Brody's form of expression seems to be slightly better. The deviations are shown graphically in Figure 4. This figure makes it clear that the computed values by the two equations are nearly the same. Davidson's

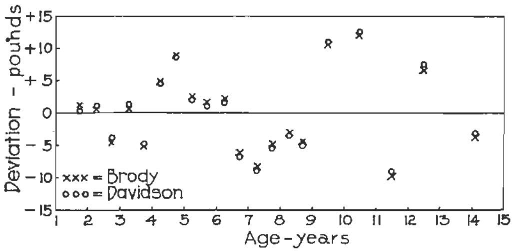

FIG. 4.—Deviation of observed weights of Jersey cows from calculated weights. The same set of observed weights of Jersey original-entry cows has been fitted with the growth equations of Brody (*1*) and Davidson (*5*). The chart shows the observed minus the calculated values

equation conforms to the observed values slightly better at the younger ages, Brody's better at the older ages. But the present method of discrimination does not show any very pronounced choice between the two forms of expression. The question seems to be, shall we say that the rate of decrease in the rate of growth is 51 per cent (Brody) or 55 per cent (Davidson) per annum?

The relation of these growth equations to (VI) and (VII) is readily apparent, and this justifies their consideration in the present connection. Equation (VI) appears to merit more attention as an expression of age changes in the rate of milk secretion. The matter is not pressed further on the basis of the present milk-yield data, however, for the reason that the cows have undoubtedly been steadily weeded out on the basis of their milk yields in previous lactations. Sanders (*14*) has used a method of eliminating, in part at least, the effect of such selection, and the curve resulting from his method is much less asymmetrical than the raw curve such as we are here dealing with. The choice between equations (VI) and (VII) might well rest, it would seem, upon the relative merits of the corresponding growth equations.

SUMMARY

This paper deals with the records of Red Danish cows published in the Danish herdbooks. The records are comparable with American Cow Testing Association records for fiscal years. The curve, due to Davidson, log $y = a - be^{-kx} - ce^{hx}$, has been fitted by the method of least squares to 4,109 annual records of the breed. The calculated maximum rate of production, 9,057 pounds of 4-per-cent milk (3,079 therms) per year is attained during the eighth year of life.

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MORPHOLOGY AND TAXONOMY OF THE PECAN-SCAB FUNGUS, CLADOSPORIUM EFFUSUM (WINT.) COMB. NOV.[1]

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INTRODUCTION

In the southeastern part of the United States the fungus hitherto known as *Fusicladium effusum* Wint. causes a widely prevalent and destructive disease, commonly called scab, on the wild and cultivated pecan (*Hicoria pecan* Brit.). The first recorded collection of the fungus was made by F. S. Earle, who collected affected leaves of the mockernut, *H. alba* (L.) Brit. (*Carya alba* (L.) K. Koch), near Cobden, Ill., October 1, 1882. This collection was sent to G. Winter, Berlin, Germany, who about three years later described (*11*)[3] the fungus as *F. effusum*. In 1888 Langlois collected at St. Martinsville, La., leaves of the pecan parasitized by a fungus that was described and named *F. caryigenum* (*6*). This locality is well within the range of the present known distribution of the pecan-scab disease. Orton (*9*) considered *F. caryigenum* Ell. and Lang. identical with *F. effusum* Wint.

Although the fungus is common on the pecan in the southeastern part of the United States, it has been found only occasionally on other species of Hicoria. During the last three years the writer has made several collections of the fungus on *H. alba* near Thomasville, Ga., and one near Charleston, S. C. Two collections[4] were made at Manhattan, Kans., on *H. cordiformis* (Wang.) Brit. (*C. amara* Nutt.). The fungus was also reported (*3*) on that host from Wisconsin. In 1926 Nolen (*8*) reported collecting the fungus in Florida on *H. aquatica* (Michx. f.) Brit.

THE FUNGUS

The fungus is strongly parasitic and invades only young or growing tissues. It attacks nuts (pl. 1, B), twigs, leaves (pl. 1, A), and catkins of the pecan, but it is known to attack only the leaves of other species of Hicoria. The exact method by which the germ tube enters the host has not been demonstrated. Under favorable con-

[1] Received for publication July 19, 1928; issued October, 1928.

[2] The writer wishes to express to M. B. Waite, Anna E. Jenkins, John W. Roberts, and J. R. Cole, Bureau of Plant Industry, U. S. Department of Agriculture, his appreciation for helpful suggestions and numerous courtesies rendered during the progress of the investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 186.

[4] Specimens in the herbarium of the Office of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture: No. 1897, Ellis and Everhart, North American Fungi, *Fusicladium effusum* Wint., on *Carya amara* Nutt., Manhattan, Kans., September, 1887; and No. 39, Kellerman and Swingle, Kansas Fungi, *Fusicladium effusum* Wint., on *Carya amara*, Manhattan, Kans., June, 1889.

Journal of Agricultural Research, Washington, D. C.

Vol. 37, No. 3
Aug. 1, 1928
Key No. G-641

ditions the incubation period may be as short as 4 to 5 days, but frequently lesions do not become noticeable until a period of 8 to 10 days has elapsed. The disease is rather superficial, extending only slightly below the epidermis. The affected tissues become black, hardened, and somewhat cracked, but show no tendency to decay unless they are later attacked by secondary invaders such as the pink-rot fungus (*Cephalothecium roseum* Cda.). On nut hulls, twigs, petioles, rachises, and leaf veins the fungus forms black stromata, which are raised somewhat above the infected host tissue.

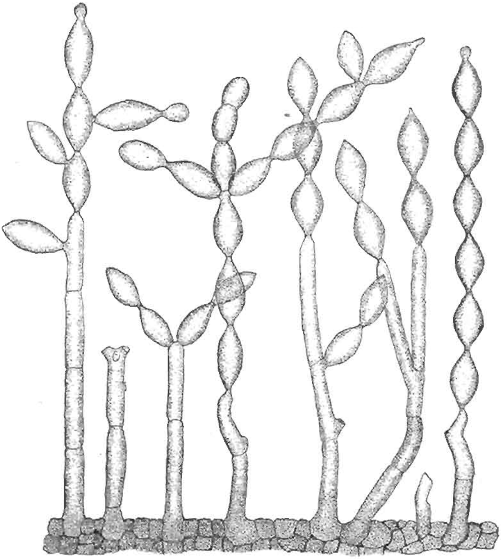
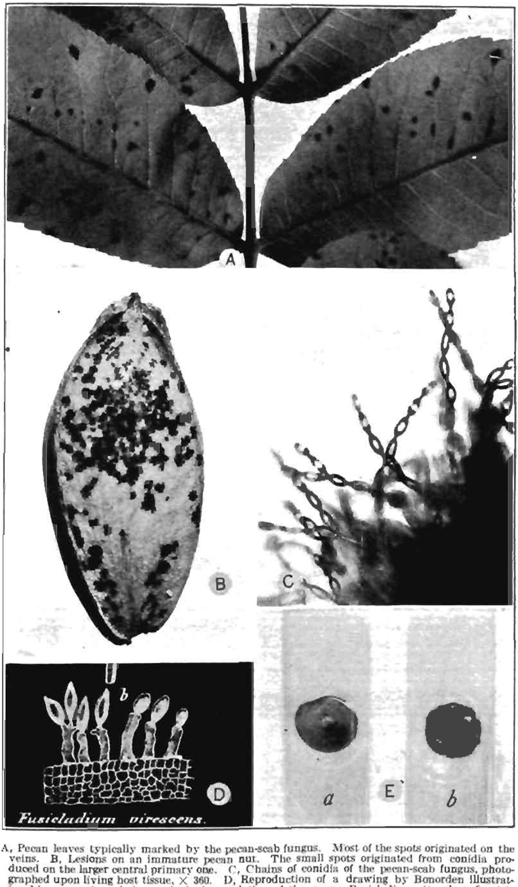


FIG. 1.—Method of sporulation and branching of the pecan-scab fungus. × about 540

The first conidiophores push out through the cuticle, but as the epidermis and the cuticle are destroyed by the anastomosing hyphae the later ones are produced directly upon the surfaces of the newly formed stromata.

The conidiophores are dark brown near the base but lighter toward the tips. They vary in length from 40 to 75 μ and may be either straight or flexuose and either simple or laterally branched. On the host tissues and under natural conditions the conidia regularly and constantly form in chains. (Pl. 1, C, and fig. 1.) The number of conidia in a chain varies from two to nine and probably averages four



A, Pecan leaves typically marked by the pecan-scab fungus. Most of the spots originated on the veins. B, Lesions on an immature pecan nut. The small spots originated from conidia produced on the larger central primary one. C, Chains of conidia of the pecan-scab fungus, photographed upon living host tissue, × 360. D, Reproduction of a drawing by Bonorden illustrating his conception of the method of sporulation of the genus Fusicladium (*1, pl. 4, fig. 94*). E, Parallel cultures of the pecan-scab fungus (*a*), and the peach-scab fungus (*Cladosporium carpophilum* Thüm. *b*), grown on Lima-bean agar for 34 days at a temperature of 24.5° C.

tissue was then sliced very thin with a hand sectioning outfit, and the sections were floated on a drop of water placed on a cover glass. Part of the water was allowed to evaporate, so that the sections would lie flat and adhere closely to the cover glass. The cover glass was then inverted over a culture ring with a drop of water in the bottom, and the joints were sealed with vaseline. Sufficient moisture to favor growth was absorbed by the host tissue and the mycelium. The specimens to be studied were then lying in a moist atmosphere and within focusing distance of a high-power objective. After exposure to a temperature of 24° to 26.5° C. for 8 to 12 hours, chains of conidia were frequently formed from the edges of the sections and in a plane parallel to the cover glass.

The technic just described permitted observation of the progress of sporulation from hour to hour and eliminated the necessity of moving the conidia from their original position during the course of the study. Cultures made by this method will remain for several days in condition for observation. Sections of living overwintered stromata from twigs or hulls will give similar results.

The catenulate arrangement of the conidia can also be demonstrated by carefully forming a crease across a new leaf infection and with the medium-power objective examining the conidia as they project beyond the leaf tissues. To get the best results from this method of observation, new leaves bearing recent infections should be collected and placed in a moist chamber for a period of 12 to 18 hours prior to examination. The chains will then stand out distinctly. Dry mounts will also show the conidia in chains, but in a manner less satisfactory for study. In 1926 the writer (*4*) called attention to the catenulate arrangement of the conidia and pointed out that this character precludes the inclusion of the fungus in the form genus Fusicladium.

The genus Fusicladium was founded by Bonorden with the species *virescens* as the type. (Pl. 1, D.) A translation of his description of the genus is as follows: "Unbranched septate hyphae, bearing at their apices, which sometimes have two projections, one or two spindle-shaped simple spores" (*1, p. 80, pl. 4, fig. 94*).

Engler and Prantl (7) list the genus Fusicladium in the Dematiaceae-didymosporae under the subdivision "Conidia not formed in chains." Saccardo (*10, p. 345*) also catalogues the genus Fusicladium in the Dematiaceae-didymosporae and under the subdivision "Conidia not catenulate—merely acrogenous."

COMPARISON WITH THE APPLE-SCAB AND PEACH-SCAB FUNGI

Fusicladium dendriticum (Wal.) Fcl., the conidial stage of *Venturia inaequalis*, has probably been studied more closely both in this country and in Europe than any other member of this form genus. In a description of this fungus Duggar (*5*) describes its method of sporulation as follows:

These conidiophores arise from the subcuticular or subepidermal mycelium * * * and a spore is soon developed at the tip of each * * *. However, when this spore is abscised, the conidiophore grows further, leaving a slight knee or other evidence indicating the point where the previous spore was borne. In this manner many successive conidia may be produced, and the conidiophore therefore becomes flexuous and irregular.

This description seems to conform quite closely to the general conception of the method by which the species of Fusicladium produce their spores.

Since the resemblance in morphological and cultural characters between the pecan-scab pathogene and the peach-scab fungus (*Cladosporium carpophilum* Thüm.) is much greater than between the former and the apple-scab fungus (*Fusicladium dendriticum*), it seemed to the writer that there might be a closer affinity between the pecan-scab and the peach-scab fungi than between the pecan-scab and the apple-scab fungi. Therefore, as a supplement to this study, comparative studies of the cultural and morphological characters of all three forms were made.

The results of these studies indicate that the pecan-scab organism possesses certain cultural and morphological characters in common with the peach-scab fungus. Both develop in artificial media very slowly at first, monosporous cultures requiring two or three weeks before becoming large enough to be seen macroscopically. Both produce a black stromatoid growth which attains a maximum surface diameter of 10 to 15 mm. in 8 to 10 weeks. Upon artificial media and living host tissues both produce, acropetally, catenulate conidia that are much alike in shape, color, and method of germination. Both produce a superficial growth upon infected host tissues, and both pass the winter as stromata or masses of pseudoparenchymatous tissue. The only essential cultural difference noted was that the pecan-scab organism produces conidia sparsely in culture, whereas young cultures of the peach pathogene produce them in abundance. (Pl. 1, E.)

Many specimens of the conidial stage of the apple-scab fungus on living apple leaves were examined. All methods were employed that had proved successful in studying the conidial production of the pecan-scab fungus, but in no certain case was the apple-scab fungus observed to be producing its conidia in chains. On the other hand, on living host tissues it regularly produces its conidia singly on short mostly one-celled sporophores in accordance with the usual conception of the genus Fusicladium.

These two forms of Fusicladium and Cladosporium were selected for comparison with the pecan fungus on account of their generally accepted validity, accessibility, and adaptibility as types for study. Furthermore, Clinton (2) thinks it possible that *F. virescens* Bon., the type species of the genus Fusicladium, may be identical with either the conidial stage of the apple-scab or the pear-scab fungus. The evidence seems to suggest that it is more probably identical with the apple-scab fungus, since Bonorden gives the apple as the host for his fungus.

The results of those comparative studies indicate that the pecan-scab organism possesses certain cultural and morphological characteristics in common with some members of the genus Cladosporium. Its similarity with *C. carpophilum* is so striking that it suggests the probability of close relationship and seems sufficient to warrant its transfer from the form genus Fusicladium to the form genus Cladosporium.

EMENDED DESCRIPTION

The reclassification of the fungus as a member of the form genus Cladosporium is proposed, with, of course, the retention of Winter's specific name. It seems desirable to include in the description of the fungus the hitherto unpublished facts concerning its morphology. The following emended description of the species is therefore given:

Cladosporium effusum, comb. nov. Syn. *Fusicladium effusum* Wint., 1885, Jour. Mycol. 1: 101. *Fusicladium caryigenum* Ell. and Lang., 1888, Jour. Mycol. 4: 124.

Mycelium olive brown, subepidermal at first, later forming pseudoparenchyma of more or less irregular cells on twigs, petioles, and fruits. Stromata formed late in summer remaining in semidormant condition during the winter and sporulating the following spring; conidiophores simple or branched, 1 to 4 septate, dark brown, 40 to 75 μ long. Conidia fusoid to ovate clavate, light olive brown, continuous, occasionally becoming 1 to 2 septate, 4.5 to 10 μ by 10 to 28 μ, catenulate, forming acropetally; chains frequently branched.

Parasitic on nut hulls, twigs, leaves, catkins of the pecan (*Hicoria pecan*), and on leaves of some other species of the genus Hicoria in the central and southeastern parts of the United States.

In artificial culture the fungus grows very slowly. Ten to fifteen days are often required for growth originating from a single conidium to become large enough to be seen macroscopically. When fully developed, which requires from two to three months, the colonies are lens shaped to irregular. The submerged portion is dark brown to black. The exposed part of the stromatoid mass is covered with brown to gray aerial hyphae. Conidia are produced but sparsely in artificial cultures.

The fungus first forms noncolored restricted areas on the host. Later the spots appear black, as the mycelium forms pseudoparenchyma or stromata involving or rupturing the epidermis.

SUMMARY

The pecan-scab organism has for many years been accepted as a species of the form genus Fusicladium. This genus, according to the description by Bonorden, its author, should include only those forms of the Dematiaceae forming one-celled conidia singly or in pairs on short conidiophores.

The result of the present study demonstrates that the pecan-scab fungus forms its conidia in either simple or branched chains. The number of conidia in a chain seems to vary from two to nine, and probably averages four or five.

Simple methods of demonstrating the catenulate arrangement of the conidia are described.

The reclassification of the fungus as a member of the form genus Cladosporium with the name *C. effusum*, comb. nov. is proposed.

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